プレニル鎖延長酵素の構造と機能

最終講義

3/6/2009

プレニル鎖延長酵素の構造と機能

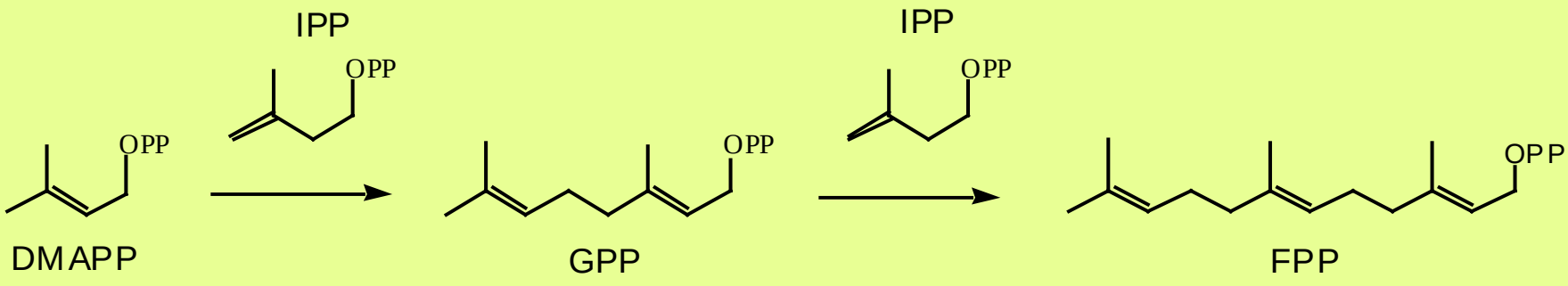
東北大学多元物質科学研究所

古 山 種 俊

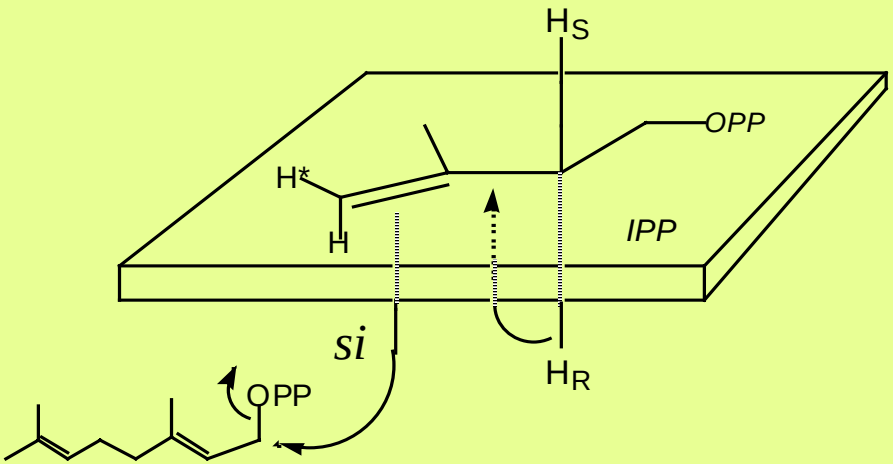
略　歴

- 1945年7月15日
- 1964年3月　静岡県立下田北高校　卒業
- 1968年3月　東北大学　理学部　化学科　卒業（田宮信雄教授）
- 1970年3月　東北大学大学理学研究科化学専攻修士課程修了（瀬戸秀一教授）
- 1973年3月　東北大学大学院理学研究科化学専攻博士課程修了・理学博士
- （瀬戸秀一教授）
- 1973年6月～1974年8月　米国 スタンフォード大学化学科博士研究員
- （E.E.van Tamelen 教授）
- 1974年9月～1991年3月　東北大学　非水溶液化学研究所　助手
- （瀬戸秀一教授：小倉 協三 教授）
- 1991年4月～1994年9月　東北大学 反応化学研究所　助手（小倉 協三 教授）
- 1994年10月～1997年4月　東北大学 工学部生物化学工学科　助教授（西野 徳三教授）
- 1997年5月～2001年3月　東北大学 反応化学研究所　教授
- 2001年4月　東北大学 多元物質科学研究所　教授（組織改組）
- 2008年3月　定年退職

プレニル鎖延長酵素 (プレニルトランスフェラーゼ)反応



FPP Synthase Reaction



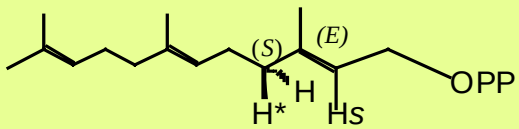
Geranyl-PP (GPP)

FPP synthase
(*rat*)

E

Reaction Stereochemistry

[J. W. Cornforth et al., 1966]



Farnesyl-PP (FPP)

立体特異的炭素 - 炭素結合形成反応

○ 人工基質に対する意外に広い基質特異性

Ogura et al. (1970); Nishino et al.,(1971); Koyama, et al., (1973) ・・・

○ 厳密な立体特異性

Ogura et al. (1974); Koyama et al. (1977); Kobayashi et al. (1985) ・・・

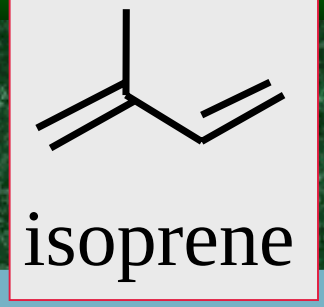
○ 昆虫幼若ホルモン類の骨格合成

JH I, JH II, JH III, JH 0, 4-MeJH I
Koyama et al. (1973); (1981); (1985); ・・・

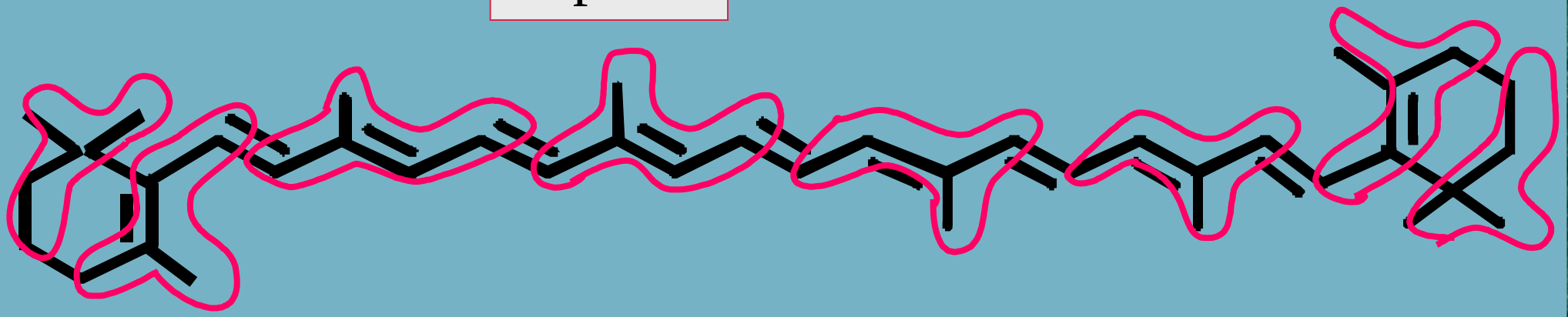
○ 昆虫フェロモンの不斉合成 Faranal

Kobayashi et al., (1980) ; Koyama et al., (1983); ・・・・

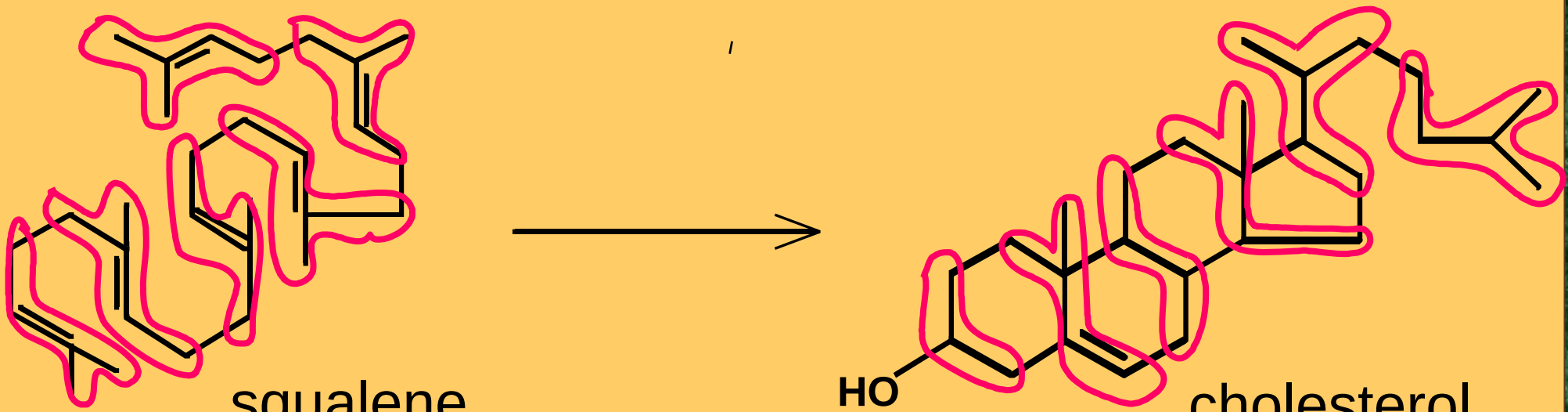
イソプレノイド：



イソプレン（C_5H_8）を構成単位とする
天然有機化合物



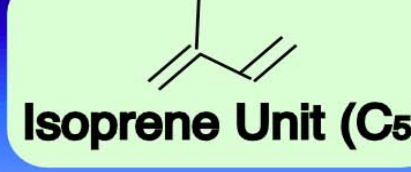
ß-carotene



squalene

cholesterol

Isoprenoid Biosynthesis



Acetyl-CoA + Acetoacetyl-CoA → HMG-CoA → Mevalonic Acid

Pyruvic Acid + Dihydroxyacetonephosphae → 1-Deoxy-D-Xylulose 5-Phosphate

Prenyltransferase Reaction

- ***E*-Prenyl Chain**
- ***Z*-Prenyl Chain**

C_5 IPP → Isopentenyl-Adenine → **Cytokinin**

C_{10} GPP → Monoterpene

C_{15} FPP → Sesquiterpene; Farnesylated Protein → **Ras, Lamin-B**

C_{30} (x 2) → Triterpene; Squalene → **Steroid**

C_{55} → **Peptideglycan**

C_{80-120} → **Glycoprotein**

$C_{>10^5}$ → **Rubber**

Betulaprenol

C_{20} GGPP → Geranylgeranylated Protein → **G-Protein**

C_{40} (x 2) → **arotenoid**; **scisic Acid, Retinoic Acid**

C_{60} → Ficaprenol

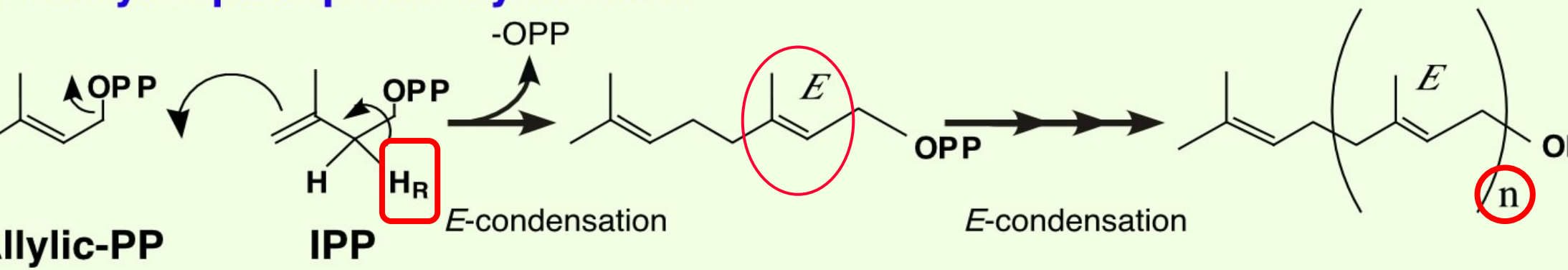
C_{25} → Diterpene → **Taxol**; Sesterterpene

C_{30} → **C_{40}** → **Vitamin K**

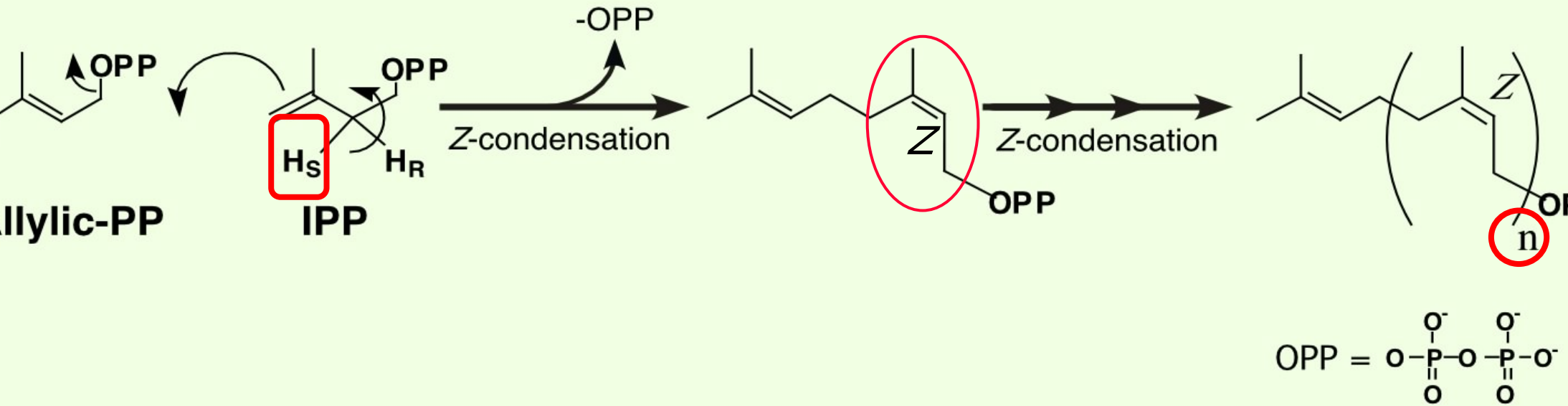
C_{50} → **C_{65}** → **Coenzyme Q**

Enzymatic Prenyl Chain Elongation

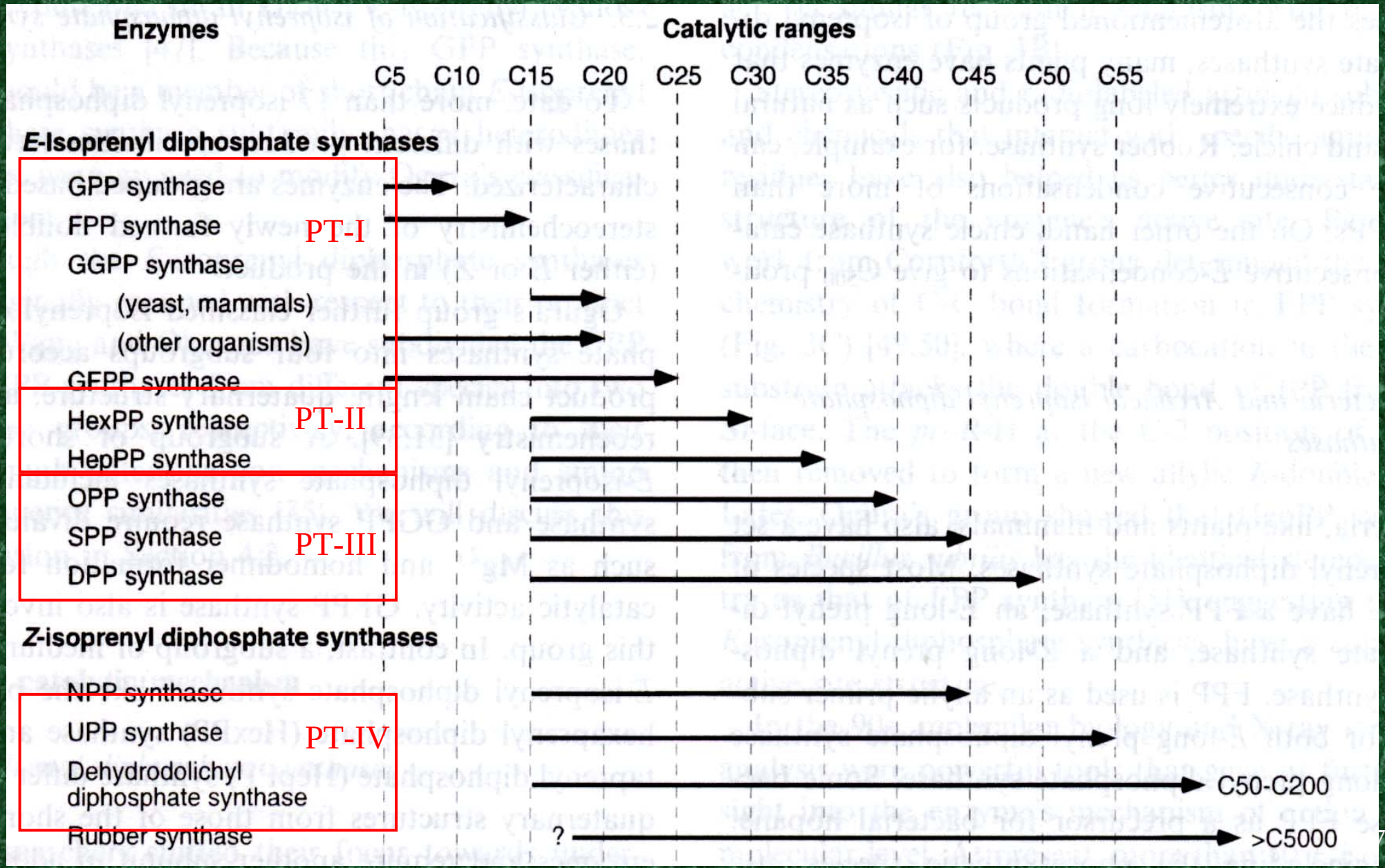
-Prenyl Diphosphate Synthases



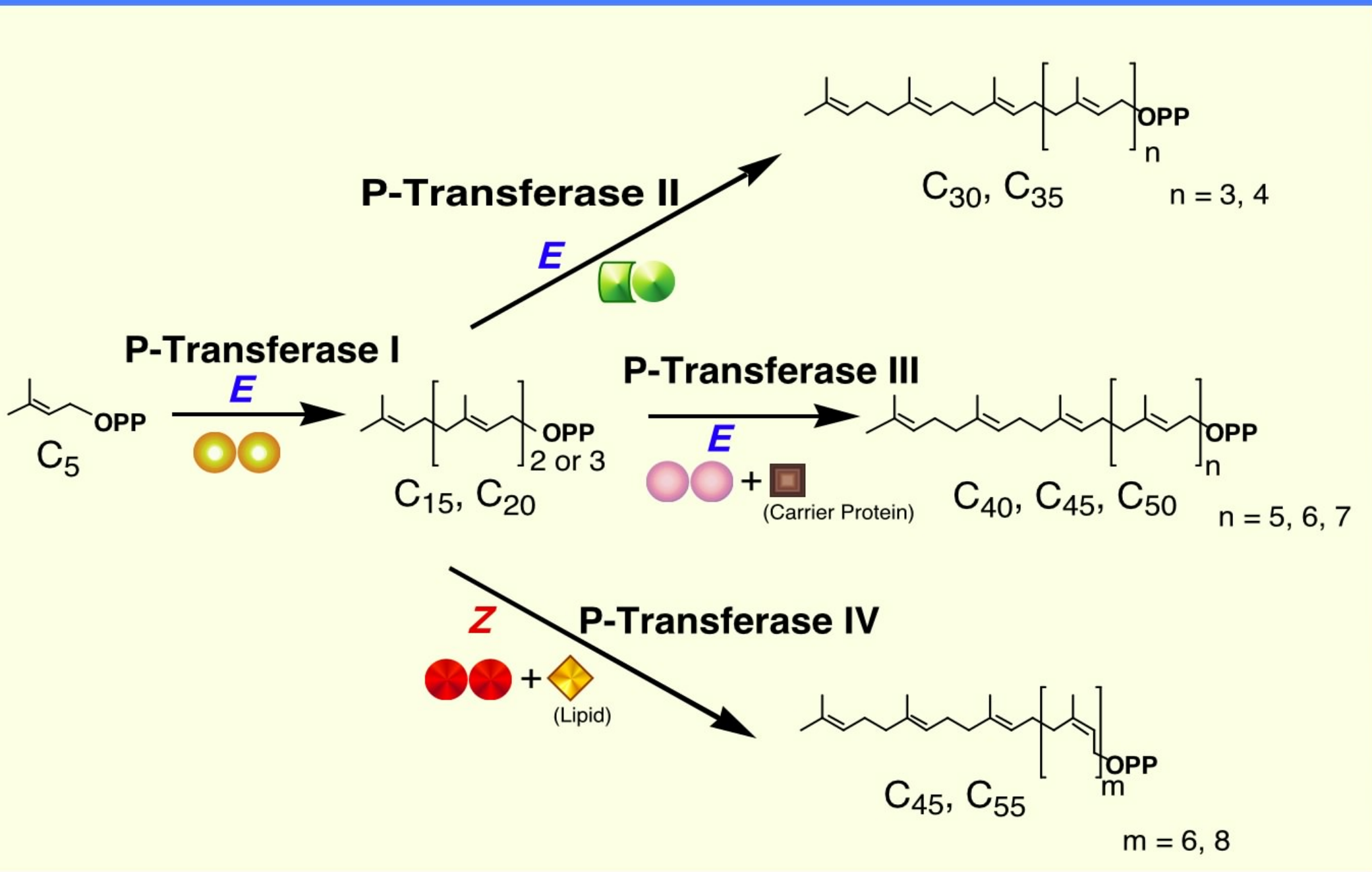
-Prenyl Diphosphate Synthases



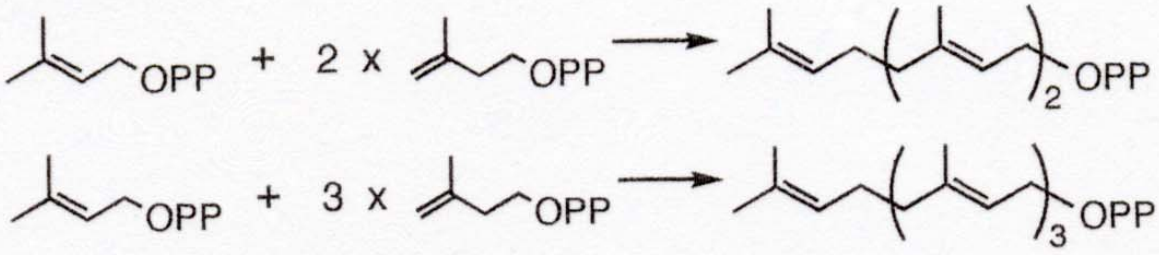
Catalytic Ranges of Prenyl Chain Elongation by Prenyltransferases so far Characterized

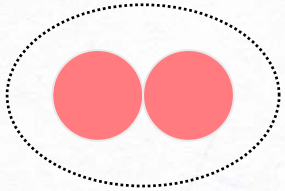


Classification of Bacterial Prenyltransferases



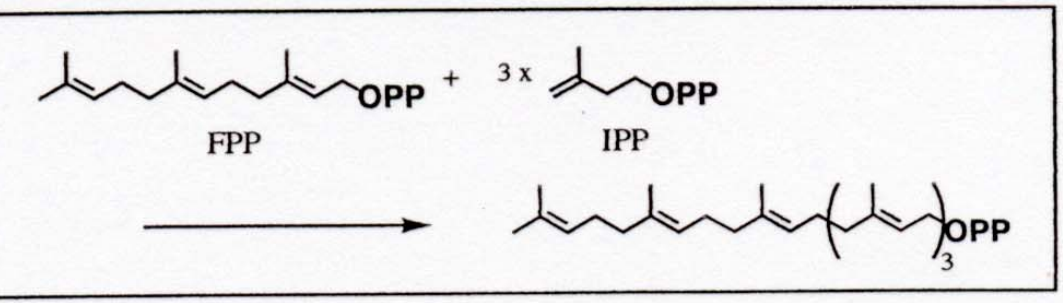
TRANSFERASE I



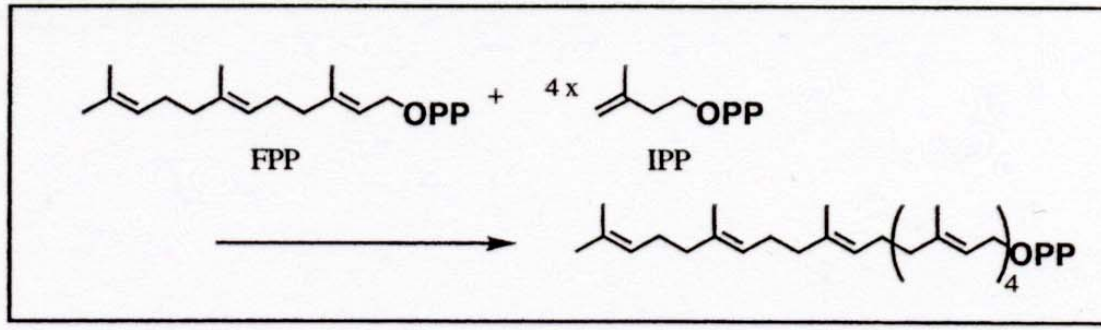


TRANSFERASE II

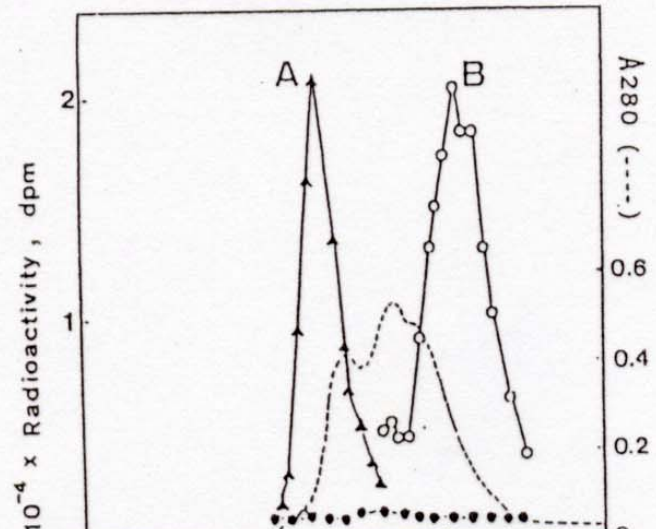
xaprenyl Diphosphate Synthase from *Micrococcus luteus* B-P 26

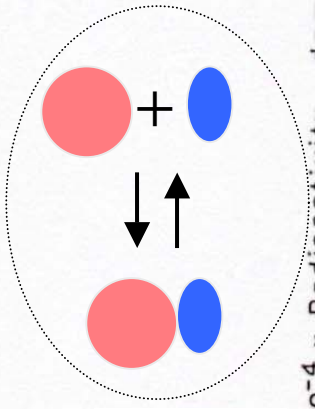


Heptaprenyl Diphosphate Synthase from *Bacillus subtilis*

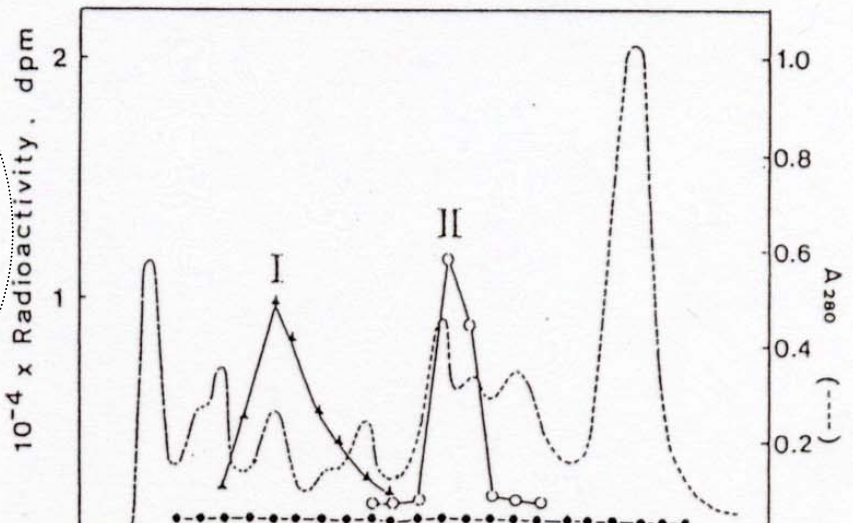


Hydroxyapatite Chromatography

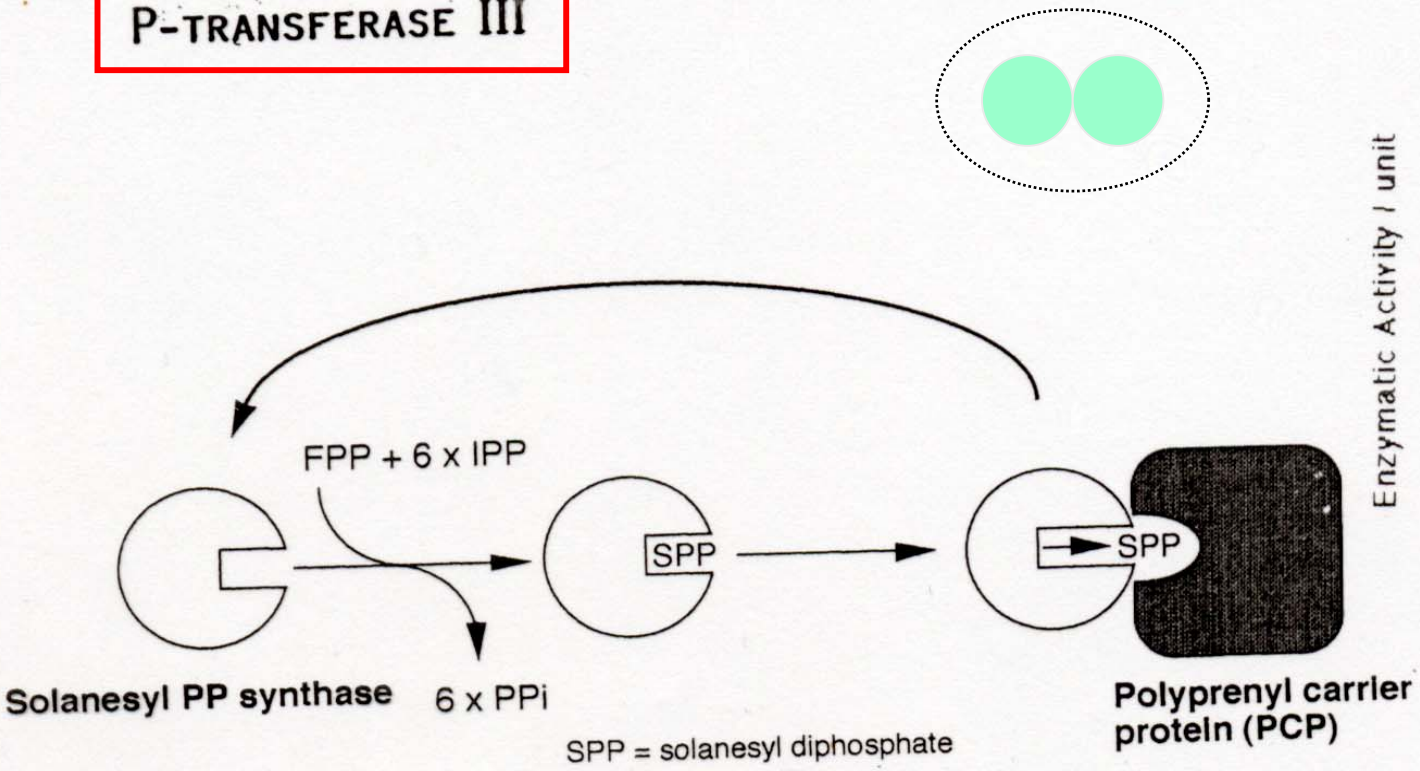




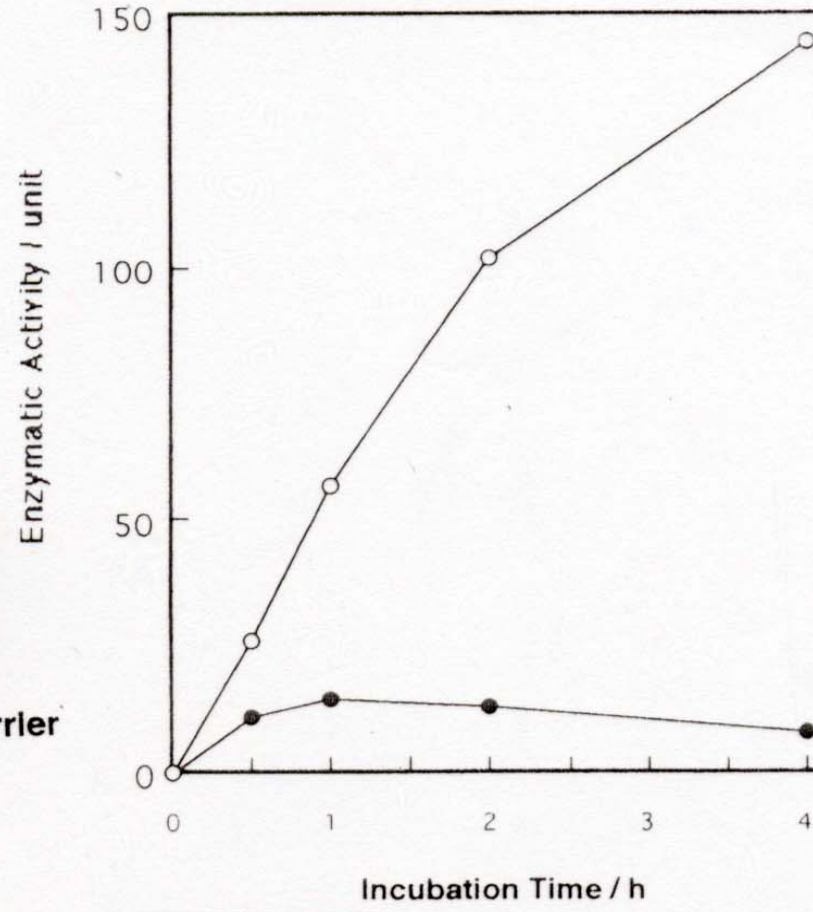
DEAE Sephadex A-50 Chromatography



P-TRANSFERASE III



Turnover of polyprenyl PP synthases dependent on PCP. SPP, Solanesyl diphosphate.



Ohnuma, S., et al.,J. Biol. Chem., 266, 22706-22713 (1991)

Molecular Analysis of Prenyl Chain Elongating Enzymes

1. *trans*-Prenyltransferases
2. *cis*-Prenyltransferases

Shotgun Cloning of *Bacilllus stearothermophilus* Farnesyl Diphosphate Synthas

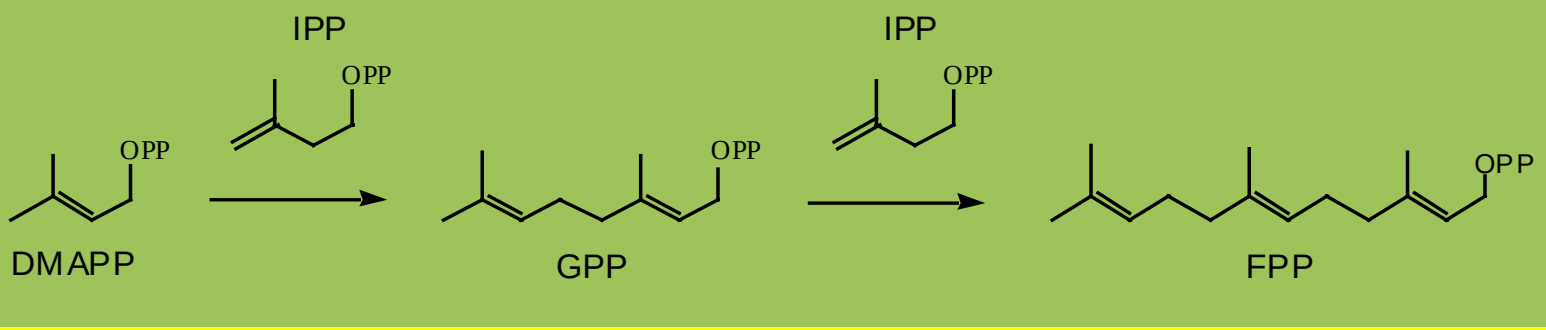
B. stearothermophilus **genomic library**

Cell-free homogenate of each transformant

Heat treatment at 55 ℃ for 30 min

Screening for heat-stable FPP synthase activity

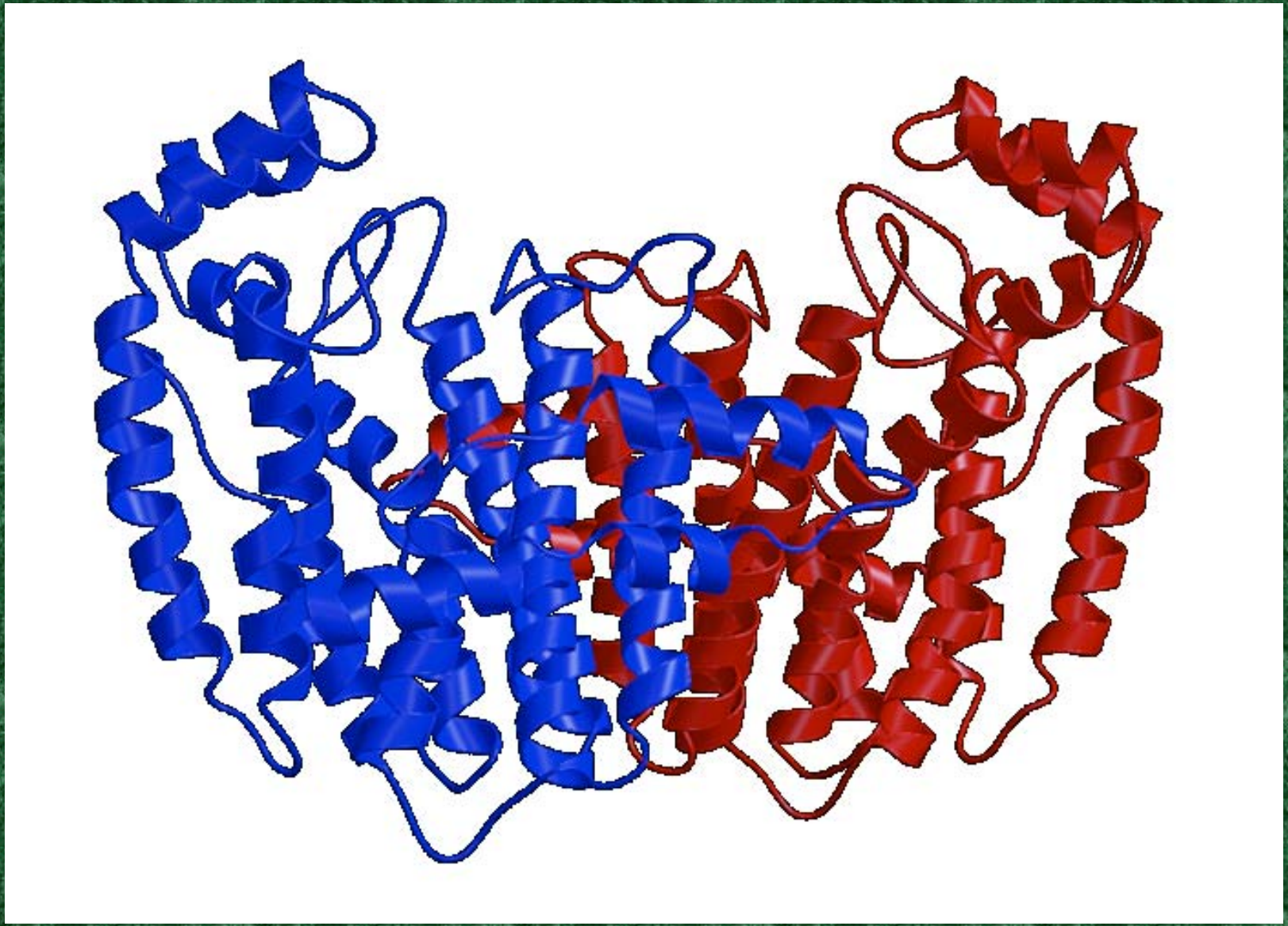
***fps* gene**



Overproduction, Purification, Crystallization

T. Koyama, S. Obata, M. Osabe, A. Takeshita, K. Yokoyama, T. Nishino, K. Ogura, *J. Biochem*., **113**, 355 (1993)

Avian Liver FPP Synthase



Tarshis, L. C. *et al*., *Biochemistry*, 33, 10871-10877 (1994)

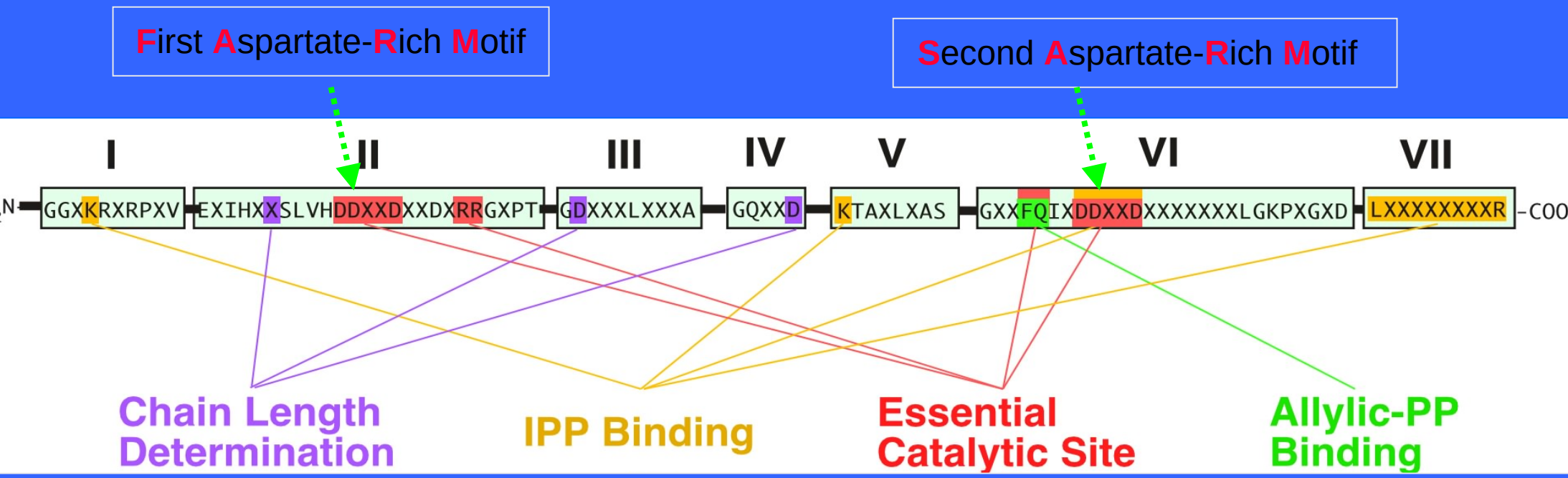
Amino acid alignments of *trans*-prenyl chain elongating enzymes

FPP synthases: B. s., *Bacillus stearothermophilus*; E. c., *Escherichia coli*; S. c., *Saccharomyces cerevisiae*; hum, human

N.c. GGPP, GGPP synthase from *Neurospora crassa*; S. c. HexPP, hexapreny-PP synthase from *Saccharomyces cerevisiae*

T. Koyama *et al.*, *J. Biochem.*, **113**, 355-363 (1993)

Roles of the Conserved Regions of *trans*-Prenyl Chain Elongating Enzymes



Koyama, T. et al., Biochemistry, 35, 9533-9538 (1996); Koyama, T. et al., Biochem. Biophys. Res. Commun., 212, 681-686 (1995).; Koyama, T. et al., Can. J. Chem., 72, 75-79 (1994); Koyama, T. et al., Biochemistry, 33, 12644-12648 (1994); Ohnuma, S. et al., J. Biol. Chem., 271, 18831-18837 (1997); Ohnuma, S. et al., J. Biol. Chem., 272, 5192-5198 (1997)

PRENYLTRANSFERASE GENES CLONED BY US

Farnesyl-PP Synthase (*Micrococcus luteus* B-P 26) (*Bacillus stearothermophilus*)

I

$C_5 \xrightarrow[(E)\text{-condensation}]{2 \times \textbf{IPP}} C_{15}$

HOMODIMER Overproduction, Purification, and Crystallization

T. Koyama, *et al.*, *J. Biochem.* **113** , 355-363 (1993); N.Shimizu *et al.*, *J. Bacteriol.*, **180**, 1578-1581 (1998).

Geranylgeranyl-PP Synthase (*Hevea brasiliensis*)

$C_5 \xrightarrow[(E)\text{-condensation}]{2 \times \textbf{IPP}} C_{20}$

HOMODIMER Overproduction, Purification

A. Takaya *et al.*, *Biochim.Biophys. Acta,* 1625, 214-220 (2003)

Hexaprenyl-PP synthase (*Micrococcus luteus* B-P 26)

II

$C_{15} \xrightarrow[(E)\text{-condensation}]{3 \times \textbf{IPP}} C_{30}$

Dissociable HETERODIMER, Overproduction, Purification Crystallization

N. Shimizu *et al.*, *J. Bacteriol.*, **180**, 1578-1581 (1998).

Heptaprenyl-PP Synthase (*Bacillus subtilis*) (*Bacillus stearothermophilus*)

$C_{15} \xrightarrow[(E)\text{-condensation}]{4 \times \textbf{IPP}} C_{35}$

Dissociable HETERODIMER, Overproduction, Purification

A. Koike-Takeshitæt *al.*, *J. Biol. Chem*, **270**, 18396-18400 (1995); Y.-W. Zhang*et al.*,*J. Bacteriol.*, **179**, 1417-1419 (1997)

Decaprenyl-PP Synthase (*Paracoccus denitrificans*)

III

$C_{15} \xrightarrow[(E)\text{-condensation}]{7 \times \textbf{IPP}} C_{50}$

HOMODIMER, Overproduction, Purification

S. Takahashi *et al.*, *Biochem. Engin. J.*, 16, 183-190 (2003)

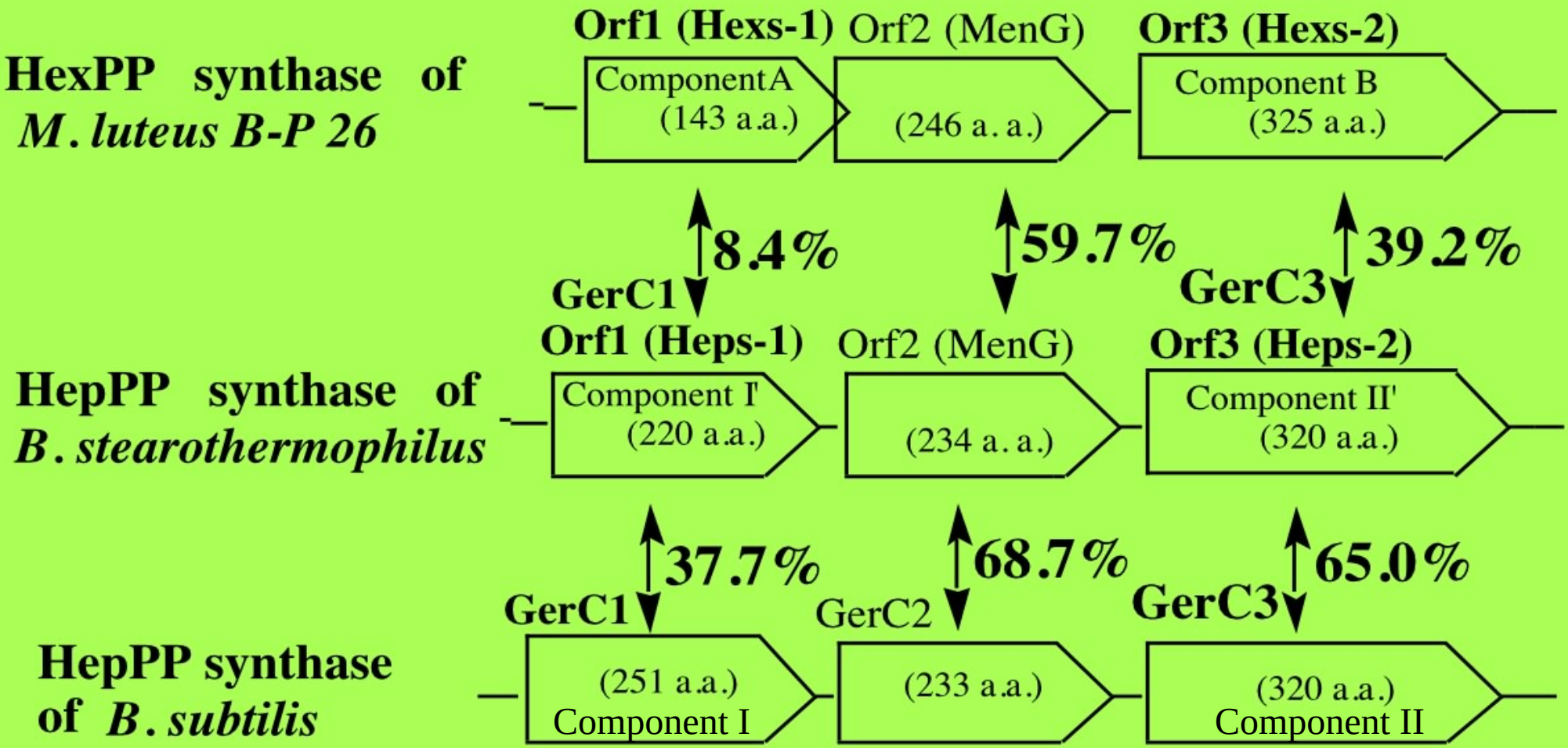
Subunit Interaction of Prenyltransferases

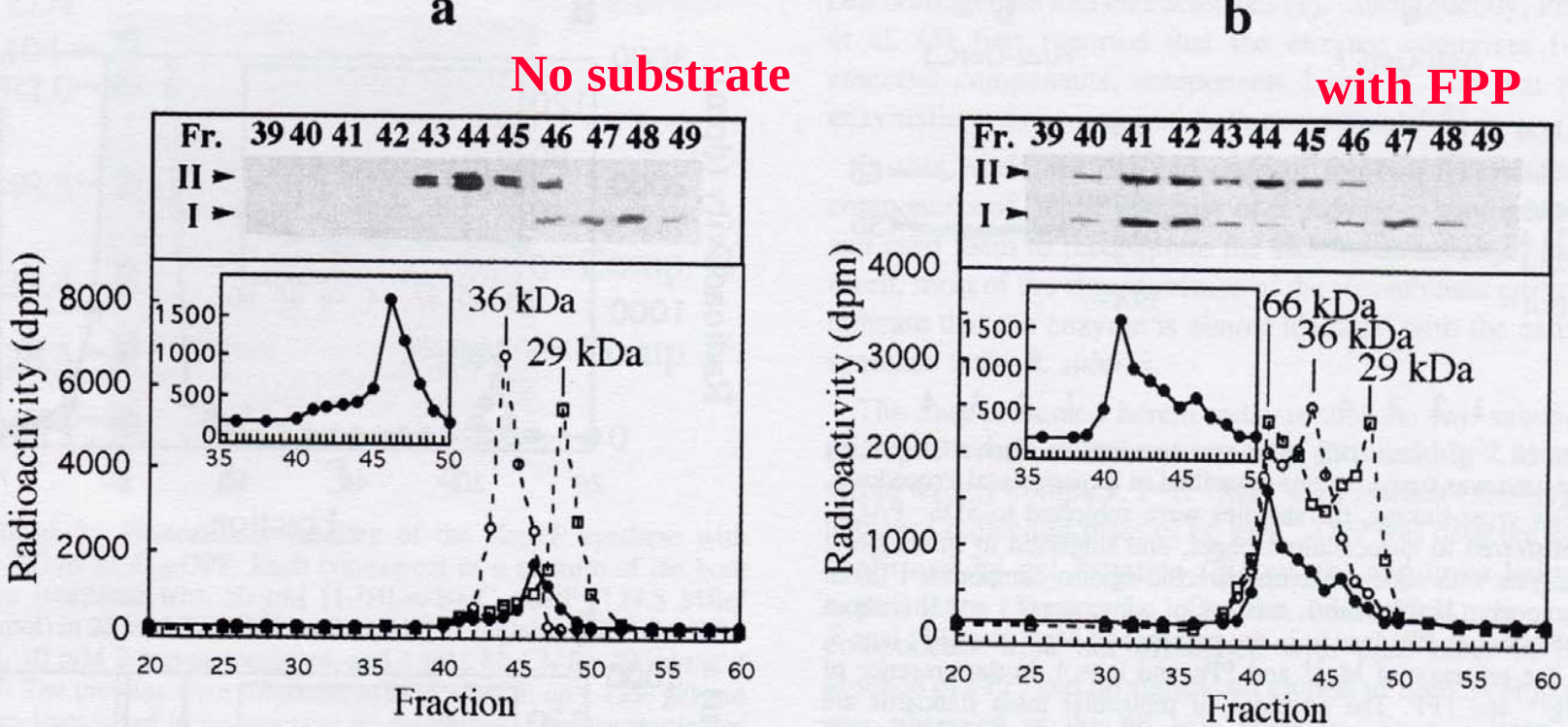
1. Dissociable heterodimers for prenyltransferase II

2. Tightly-bound homodimers for prenyltransferase I and -III

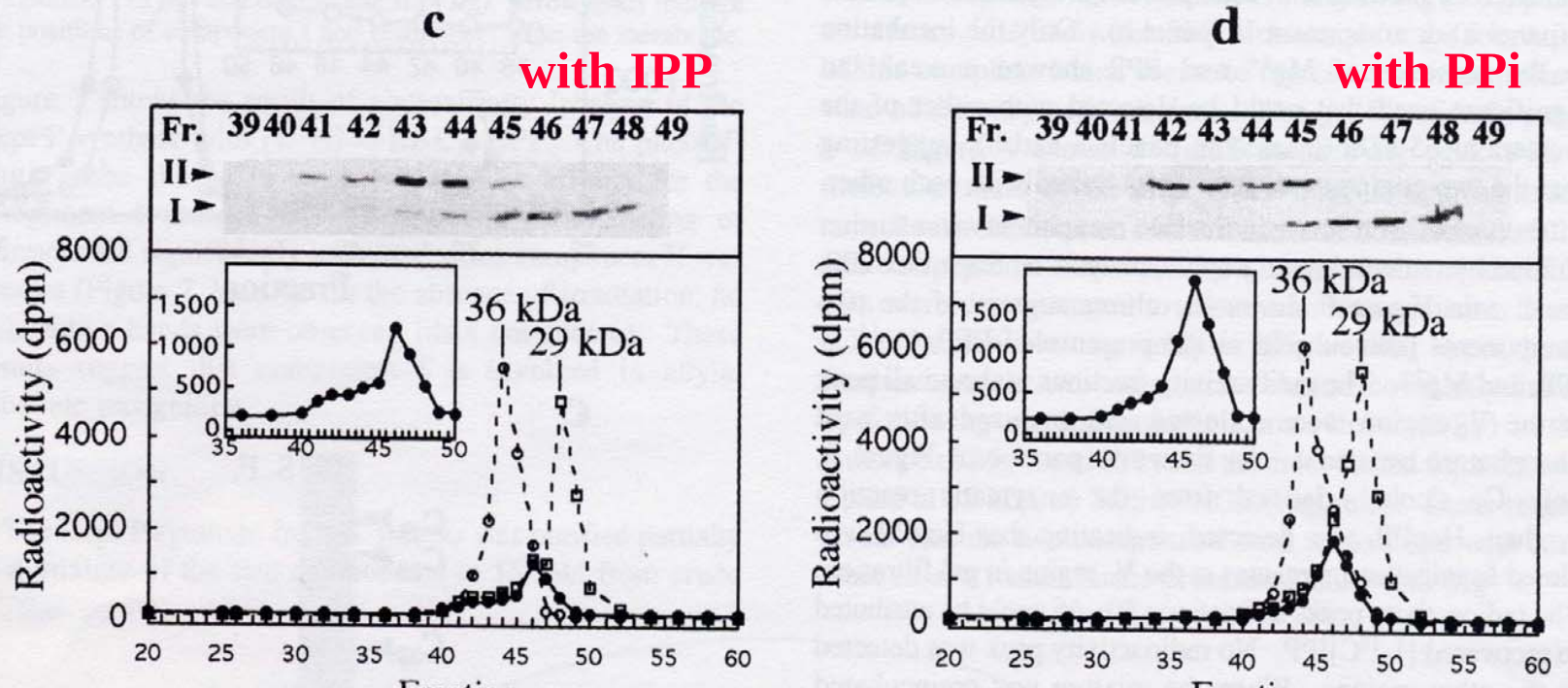
Prenyltransferase II

(*E*)-Medium-chain Prenyl Diphosphate Synthase Genes

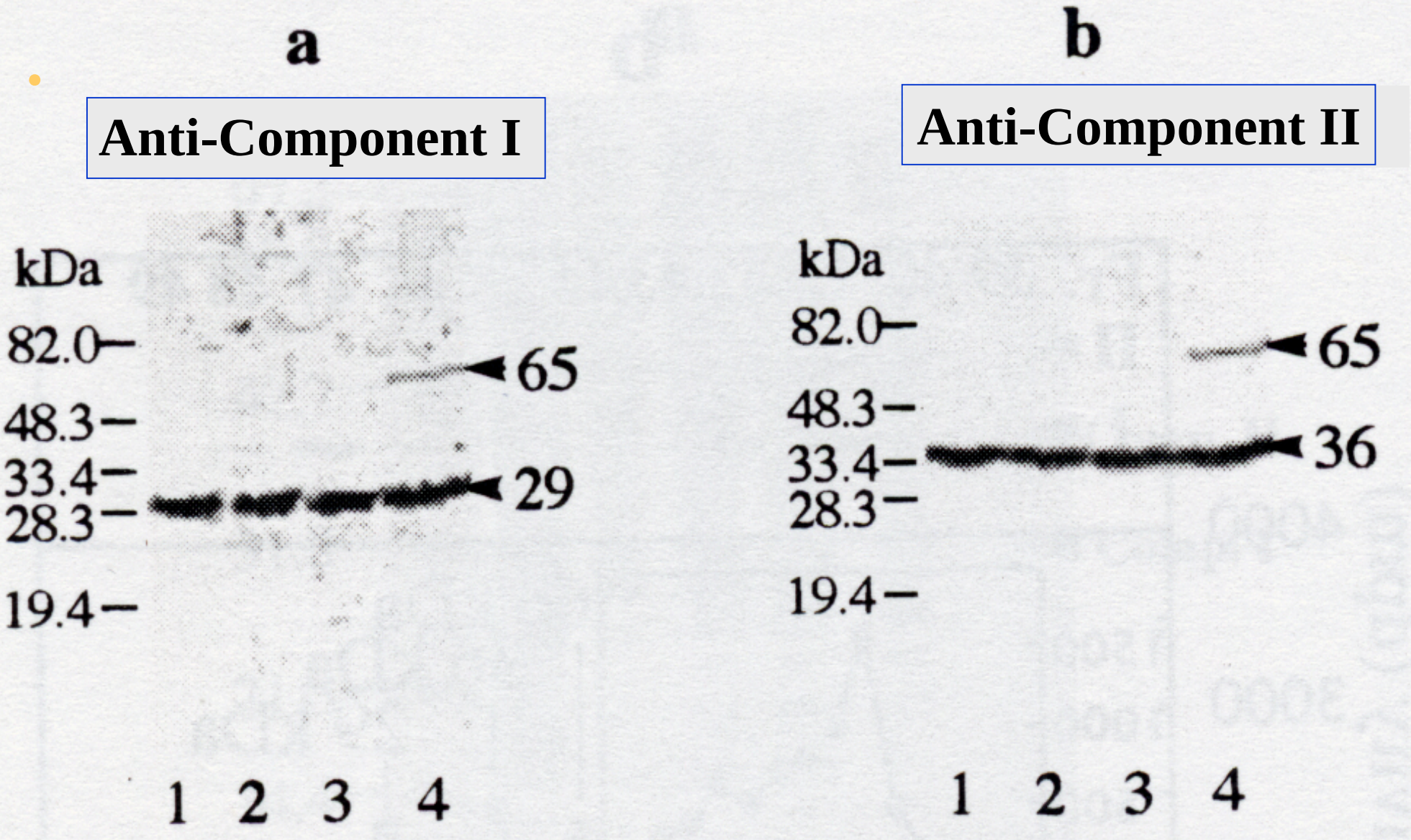




Superdex 200 Gel Filtration Chromatography



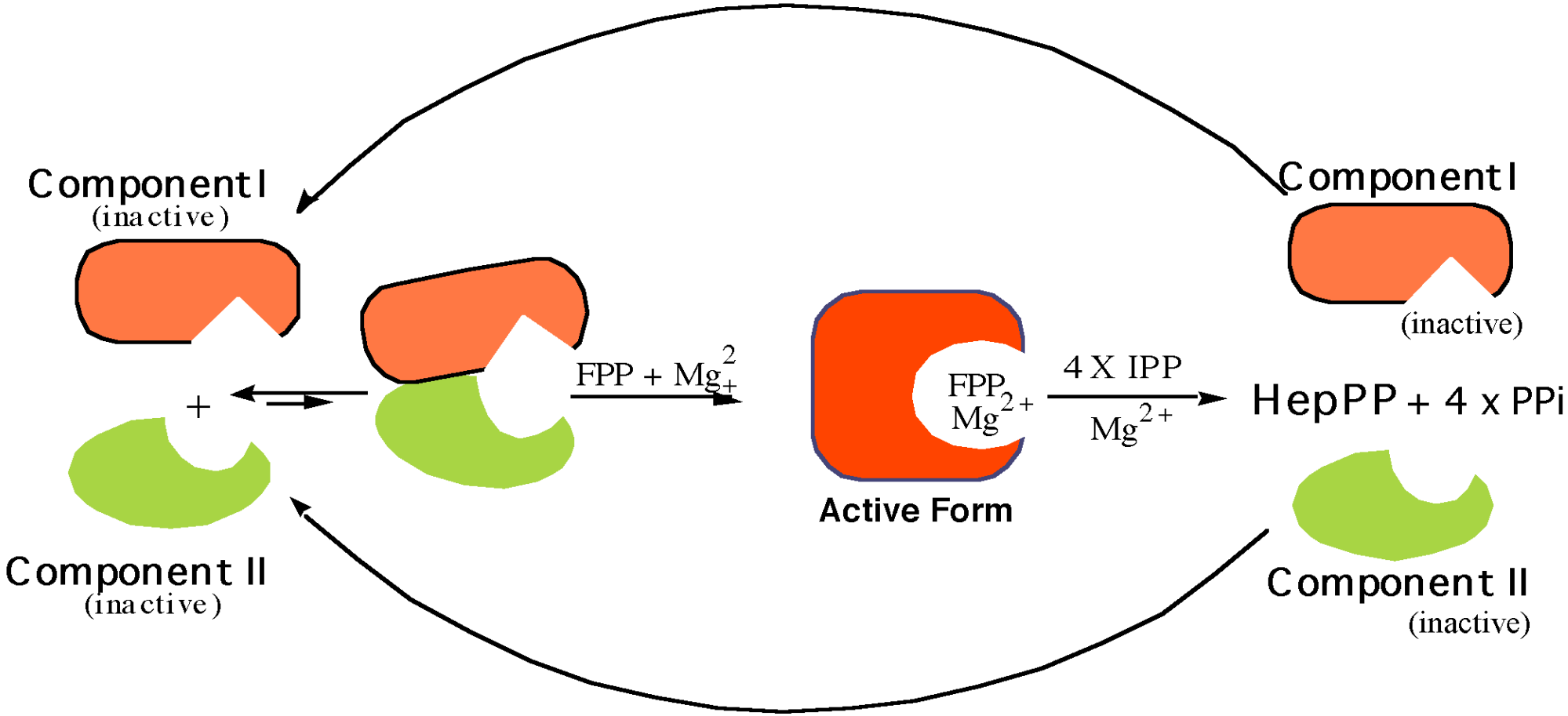
Cross-linking of the two components with disuccinimidyl suberate



(1, with no substrate; 2, with IPP; 3, with PPi; 4, with FPP)

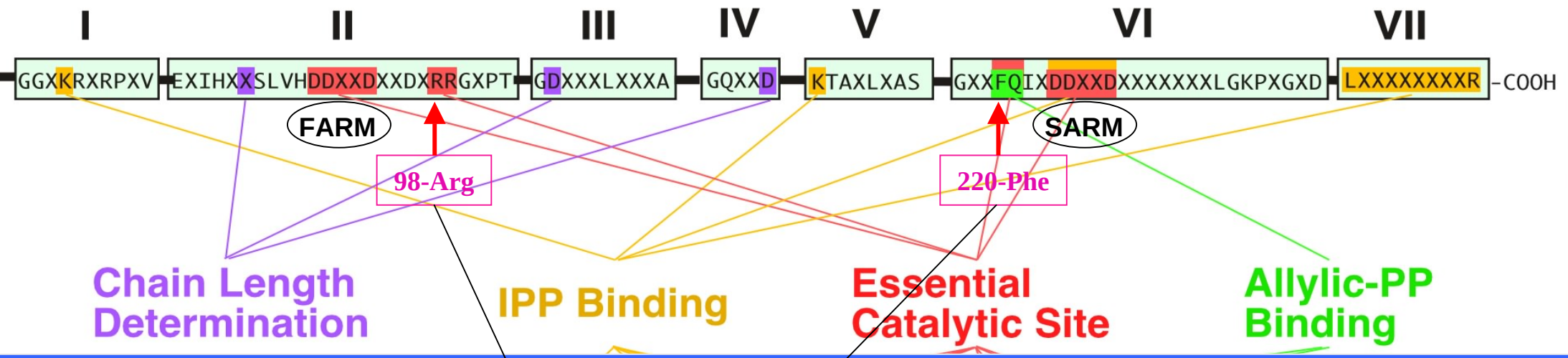
Zhang, Y-W. et al., *Biochemistry*, 37, 13411-13420 (1998)

Hypothetical Mechanism of Hep-PP Synthase Reaction

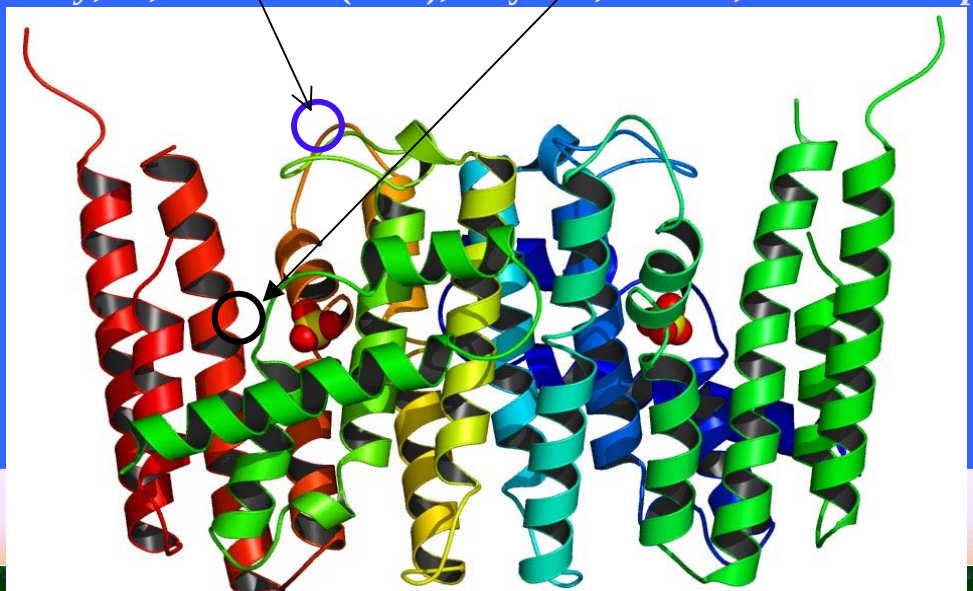


Amino Acids Crytical for the Enzymatic Function

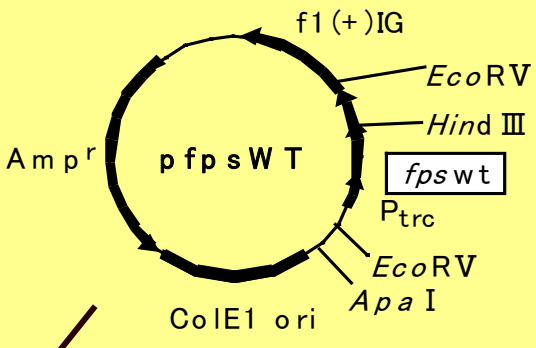
E-Prenyl Diphosphate Synthases

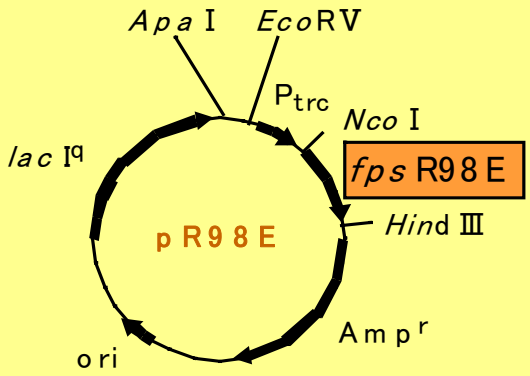


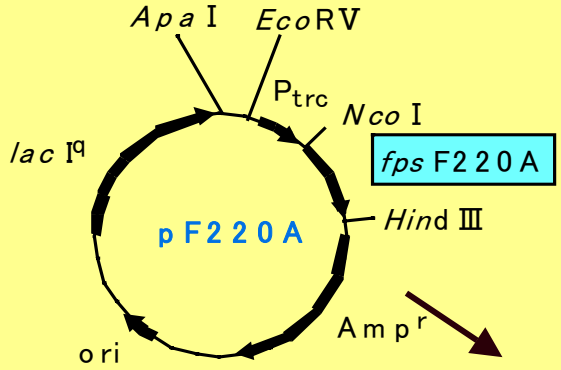
Koyama, T. et al., *Biochemistry*, 35, 9533-9538 (1996); Koyama, T. et al., *Biochem. Biophys. Res. Commun.*, 212, 681-686 (1995).



Preparation of Hybrid-Type FPP Synthase Heterodimers



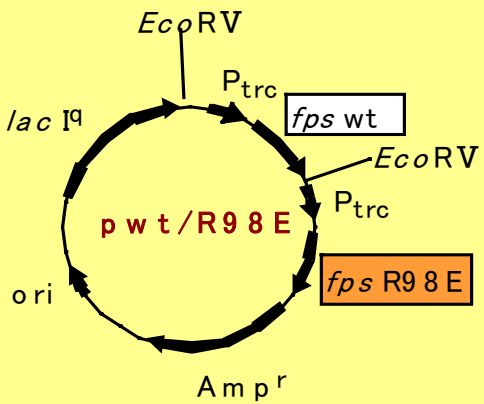


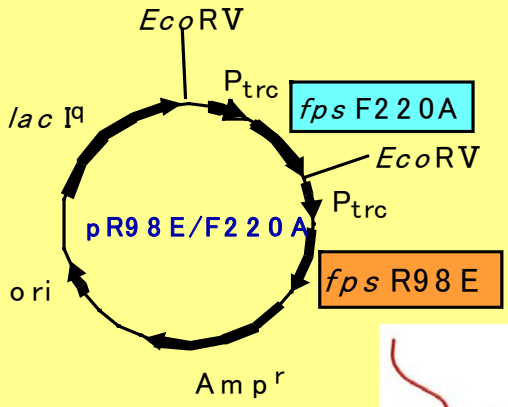


WT/WT

R98E/ R98E

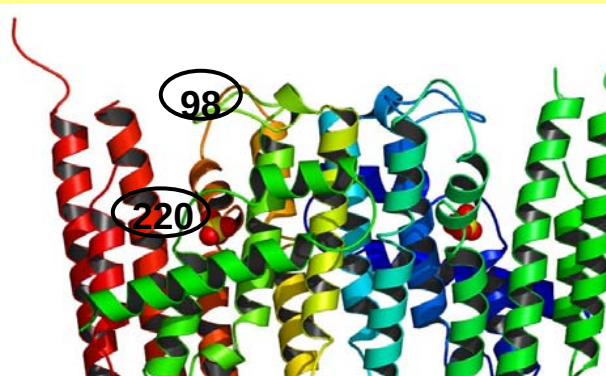
F220A/F220A





WT/ R98E

R98E/ F220A



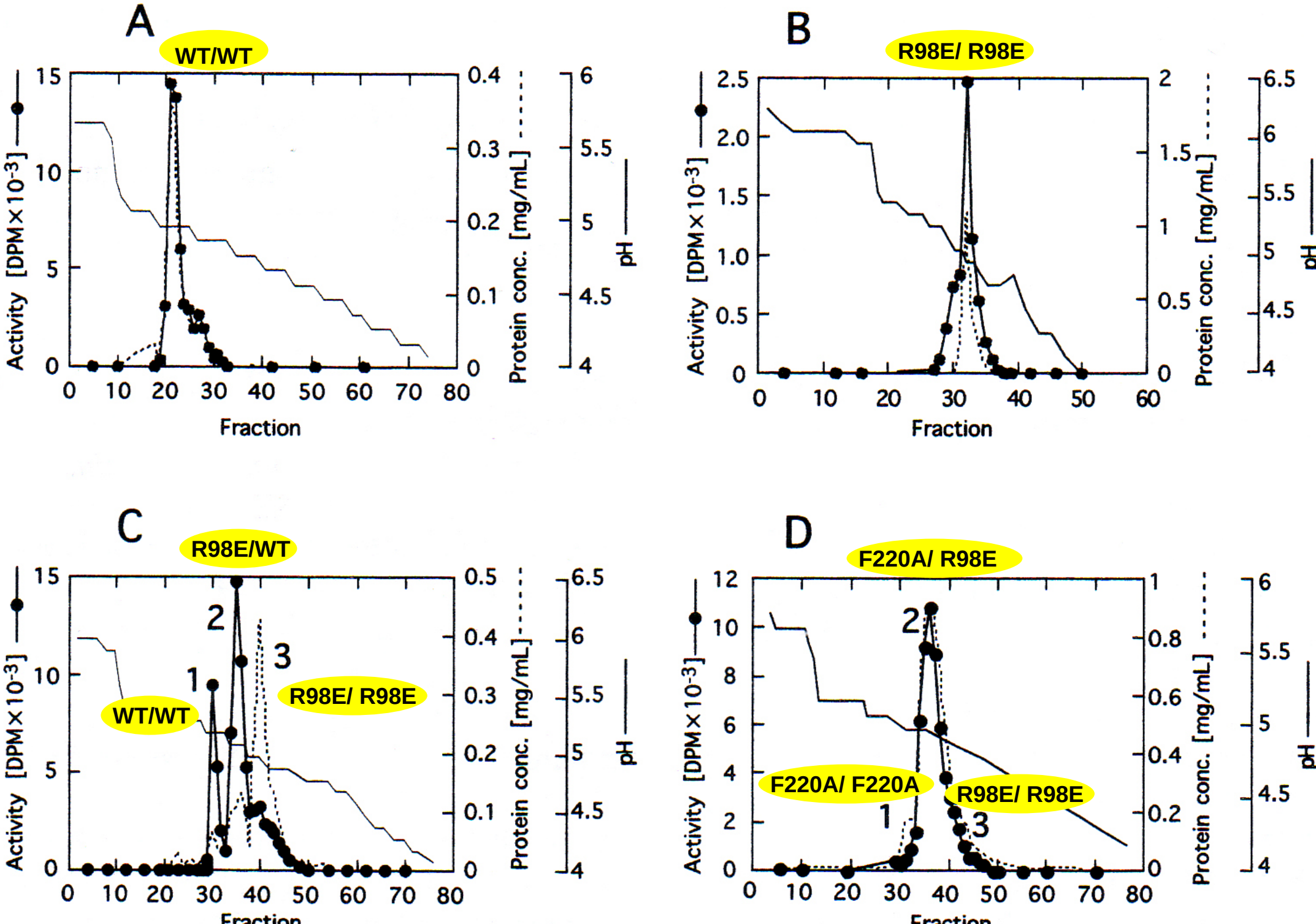
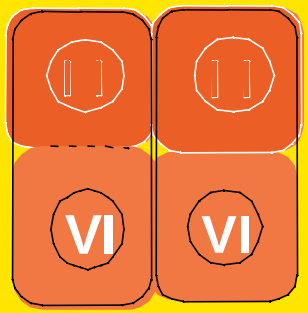

A
WT/WT
Activity [DPM×10⁻³]
Protein conc. [mg/mL]
pH
Fraction
B
R98E/ R98E
C
R98E/WT
WT/WT
R98E/ R98E
1
2
3
D
F220A/ R98E
F220A/ F220A
R98E/ R98E

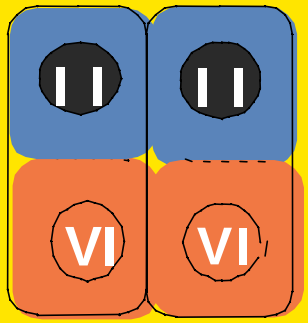
Table 1: Kinetic Parameters of Wild-Type and Mutant Homo- and Heterodimeric FPP Synthases[a]

FPP synthase	K_m(IPP)[b] (μM)	K_m(GPP) (μM)	K_m(DMAPP) (μM)	k_{cat}[c] $\times 10^4$ (s^{-1})	k_{rel}[d]
wild-type, $(WT)_2$	9.10 ± 1.23	5.54 ± 1.19	11.1 ± 1.52	90700 ± 3800	1
$(R98E)_2$	20.1 ± 2.93	12.4 ± 0.64	27.6 ± 0.20	26.3 ± 0.8	2.90×10^{-4}
$(F220A)_2$[e]	24.9 ± 3.10	3.5 ± 0.70	22.5 ± 8.6	1.18 ± 0.11	1.30×10^{-5}
WT/R98E	11.6 ± 3.21	13.5 ± 0.02	19.1 ± 0.75	71100 ± 4000	0.784
R98E/F220A	28.5 ± 5.37	9.84 ± 0.57	17.7 ± 2.40	466 ± 6.12	5.14×10^{-3}

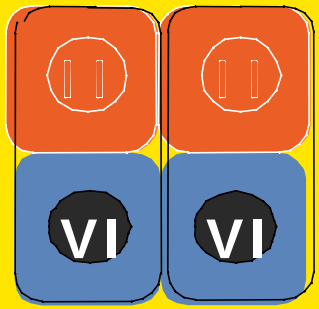
[a] Each value represents the mean $\pm$ the standard deviation of two determinations. [b] For the reaction with GPP. [c] k_{cat} was calculated from the V_m value for the reaction with IPP and GPP. [d] k_{cat} value relative to that of the wild type. [e] Data from ref 22.



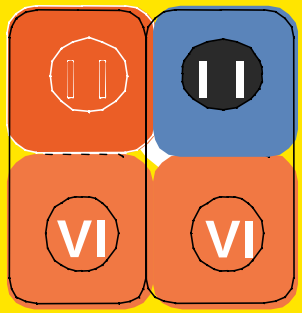
WT/WT
[k_{cat}: 9.07]



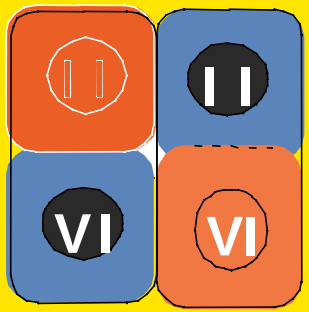
R98E/R98E
[k_{cat}: 0.0026]



F220A/F220A
[k_{cat}: 0.00011]

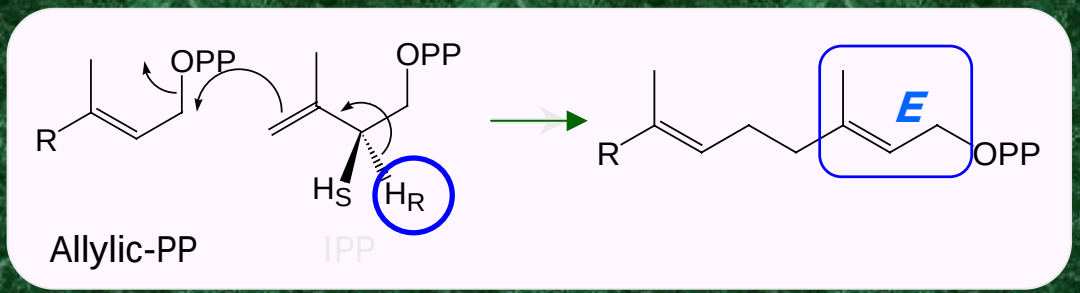


WT/R98E
[k_{cat}: 7.11]

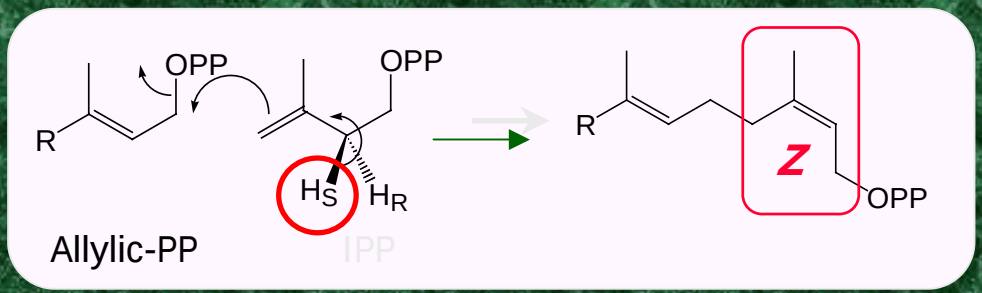


R98E/F220A
[k_{cat}: 0.047]

Cloning of Undecaprenyl Diphosphate Synthase Gene as *cis*-Prenyl Chain Elongating Enzymes



***E*(*trans*)-Prenyltransferase**



***Z*(*cis*)-Prenyltransferase**

PRENYLTRANSFERASE GENES CLONED BY US

Farnesyl-PP Synthase (*Micrococcus luteus* B-P 26) (*Bacillus stearothermophilus*)

I

$C_5 \xrightarrow[(E)\text{-condensation}]{2 \times \textbf{IPP}} C_{15}$

HOMODIMER Overproduction, Purification, and Crystallization

T. Koyama, *et al.*, *J. Biochem.* **113** , 355-363 (1993); N.Shimizu *et al.*, *J. Bacteriol.*, **180**, 1578-1581 (1998).

Geranylgeranyl-PP Synthase (*Hevea brasiliensis*)

$C_5 \xrightarrow[(E)\text{-condensation}]{2 \times \textbf{IPP}} C_{20}$

HOMODIMER Overproduction, Purification

A. Takaya *et al.*, *Biochim.Biophys. Acta,* 1625, 214-220 (2003)

Hexaprenyl-PP synthase (*Micrococcus luteus* B-P 26)

II

$C_{15} \xrightarrow[(E)\text{-condensation}]{3 \times \textbf{IPP}} C_{30}$

Dissociable HETERODIMER, Overproduction, Purification Crystallization

N. Shimizu *et al.*, *J. Bacteriol.*, **180**, 1578-1581 (1998).

Heptaprenyl-PP Synthase (*Bacillus subtilis*) (*Bacillus stearothermophilus*)

$C_{15} \xrightarrow[(E)\text{-condensation}]{4 \times \textbf{IPP}} C_{35}$

Dissociable HETERODIMER, Overproduction, Purification

A. Koike-Takeshitæt *al.*, *J. Biol. Chem*, **270**, 18396-18400 (1995); Y.-W. Zhang*et al.*,*J. Bacteriol.*, **179**, 1417-1419 (1997)

Decaprenyl-PP Synthase (*Paracoccus denitrificans*)

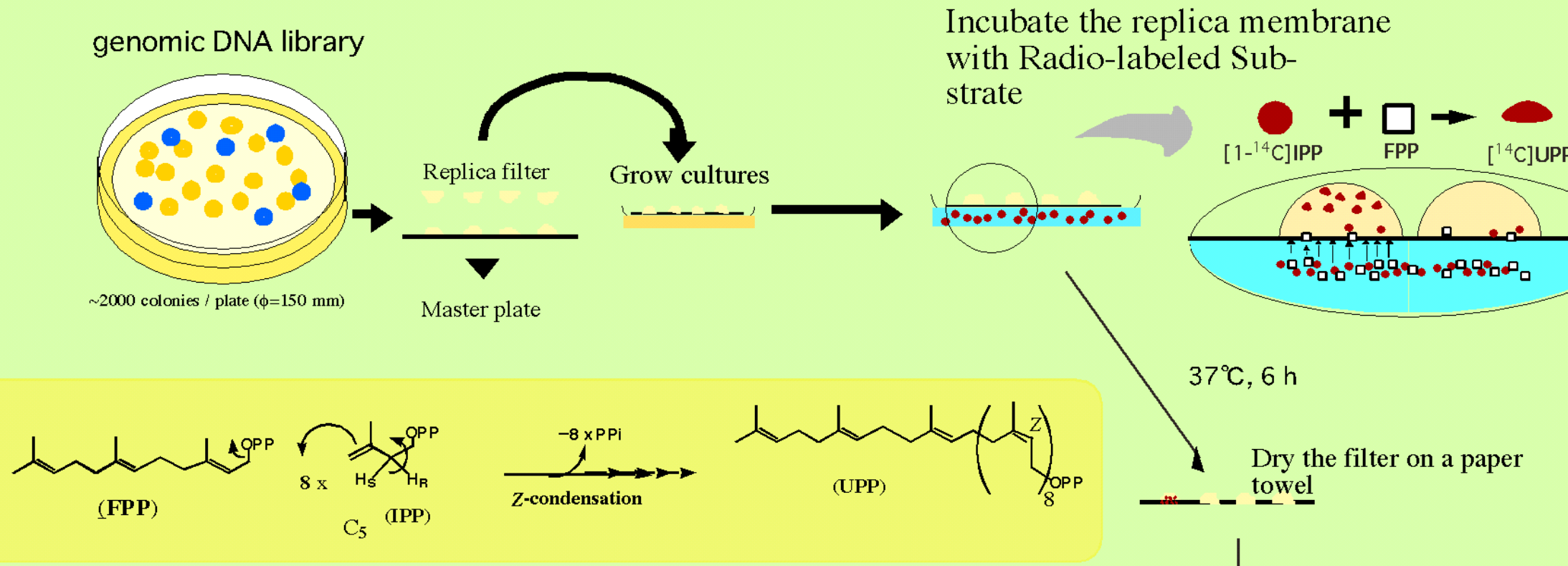
III

$C_{15} \xrightarrow[(E)\text{-condensation}]{7 \times \textbf{IPP}} C_{50}$

HOMODIMER, Overproduction, Purification

S. Takahashi *et al.*, *Biochem. Engin. J.*, 16, 183-190 (2003)

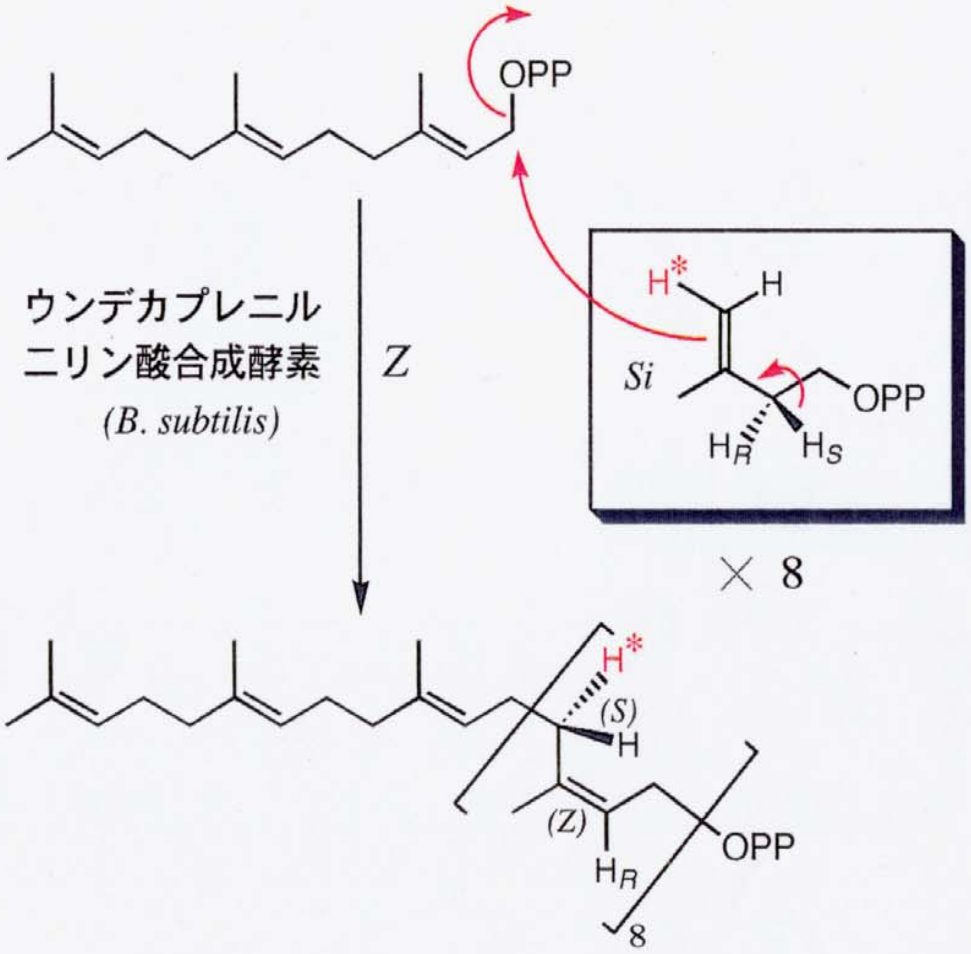
UPPSynthase Gene Cloning by Colony Autoradiography Method



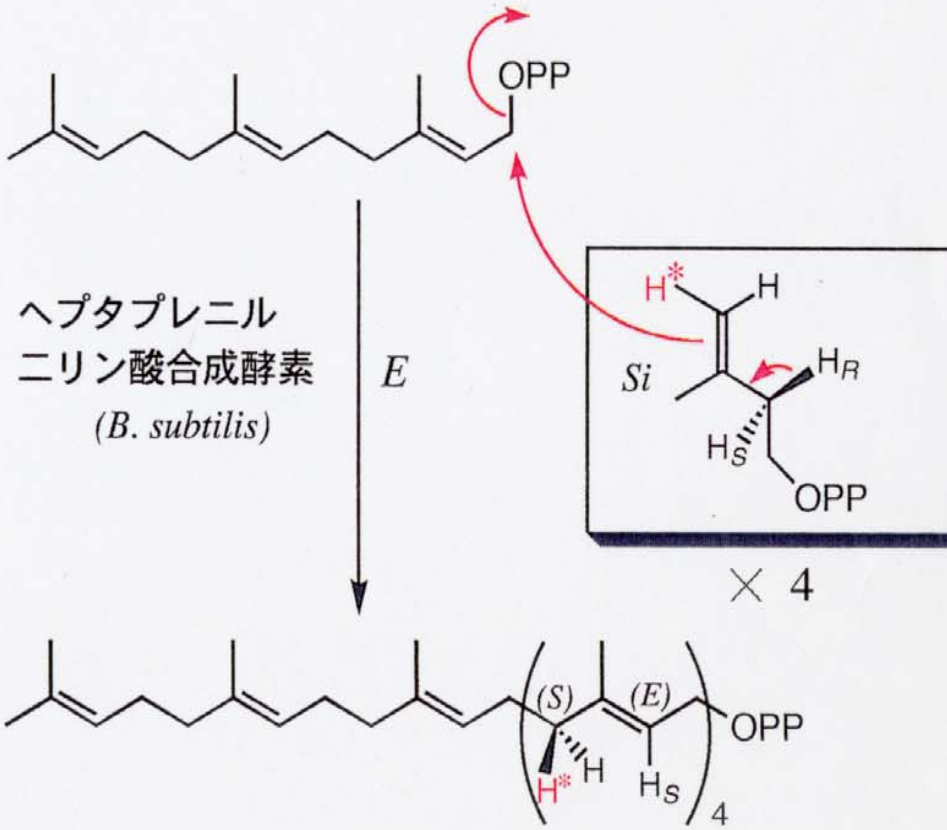
cf. C. R. H. Raetz, *Proc. Nat. Acad. Sci., USA*, **72**, 2272 (1978)

N. Shimizu *et al.*, *J. Biol. Chem.*, **273** (31) 19476-19481 (19

Z 型鎖延長反応



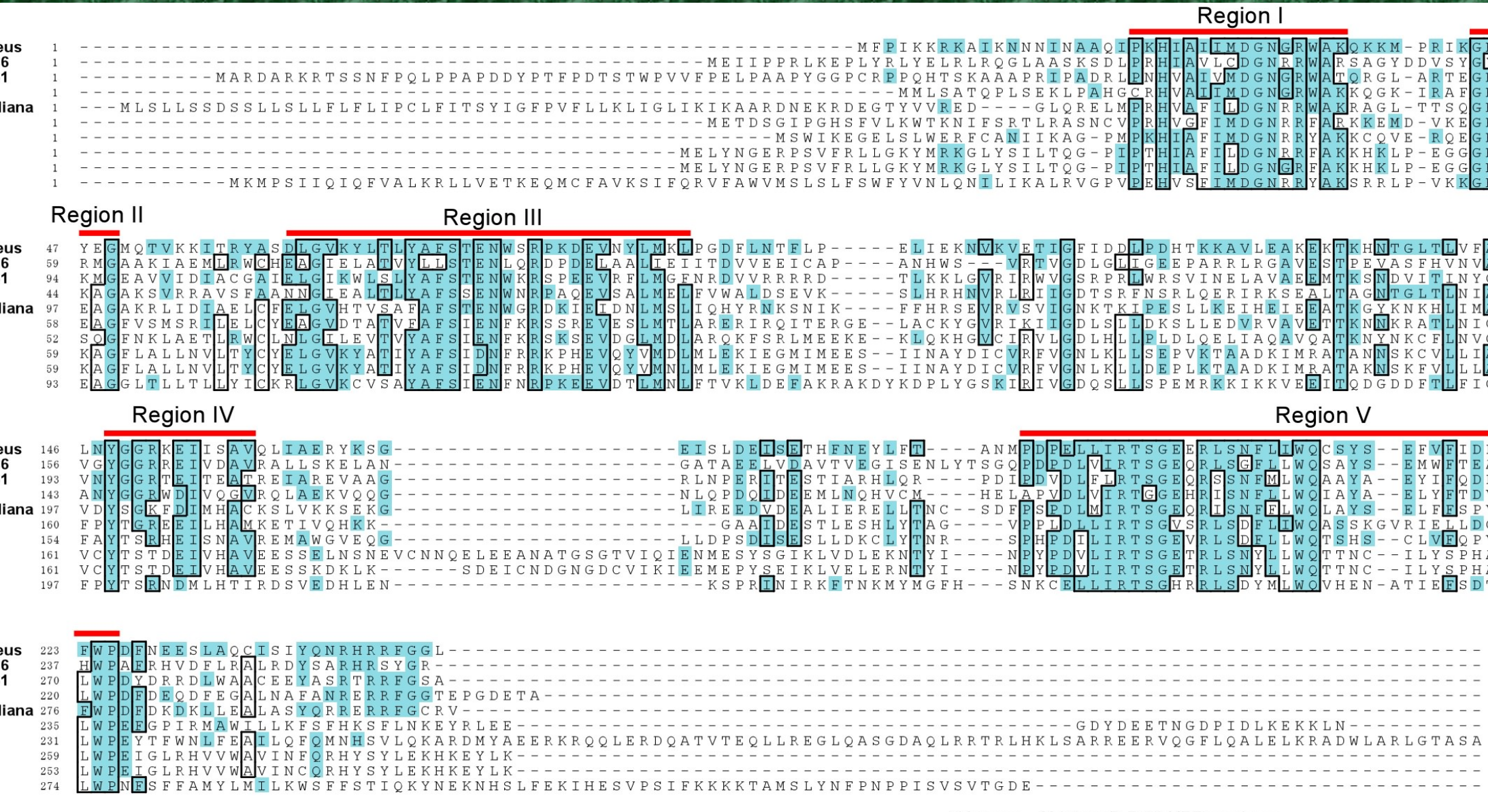
E 型鎖延長反応



Kobayashi, M. *et al.*, *J. Am. Chem. Soc.*, 107,4588-4589 (1985)

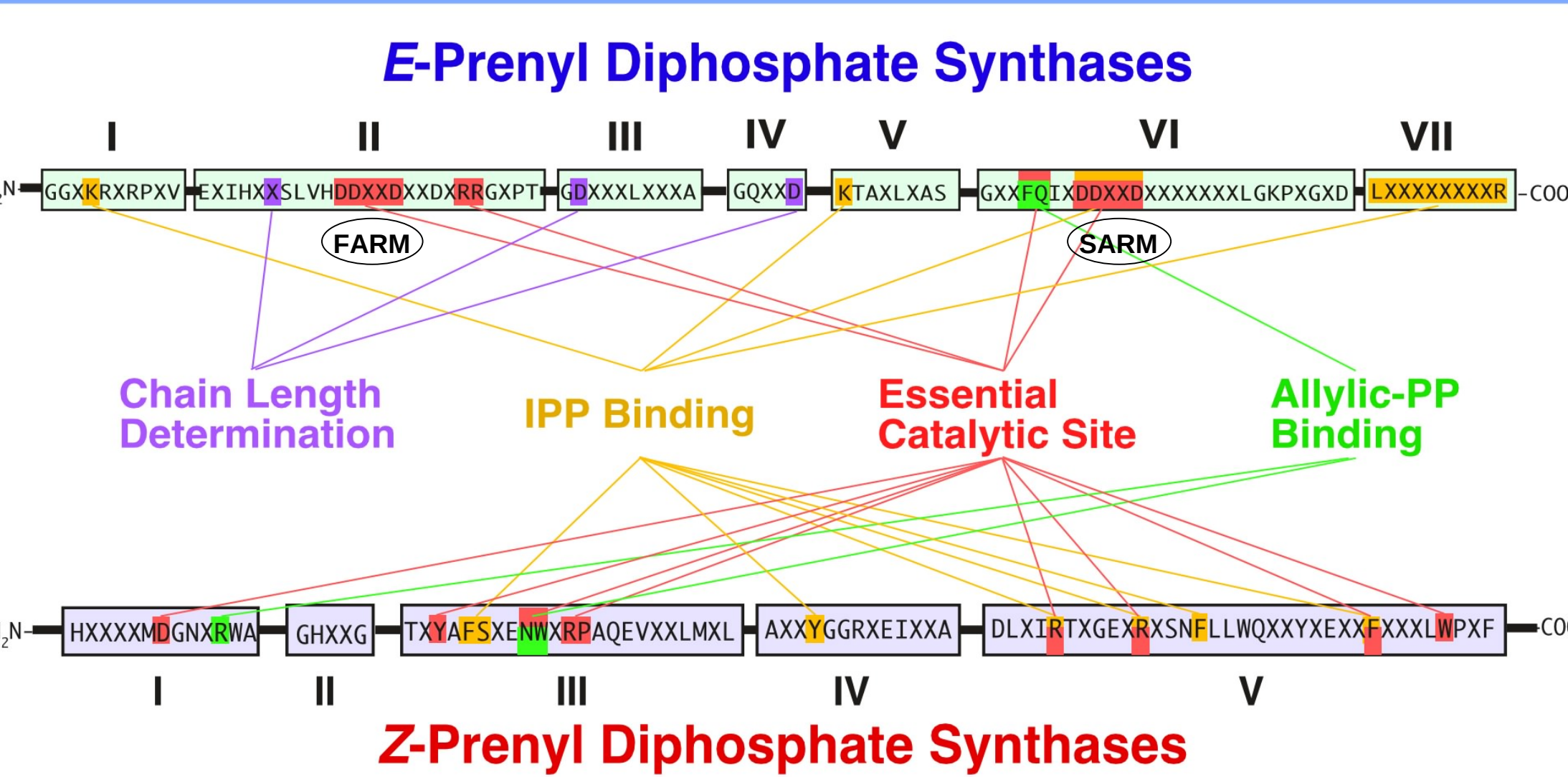
Ito, M. *et al.*, *Biochemistry*, 26, 4745-4740 (1987)

Amino acid alignments of *cis*-prenyl chain elongating enzymes



M.luteus: *M.luteus* B-P 26 UPP synthase
Rv1086: *M. tubercilosis* Rv1086 (FPP synthase)
Rv2361: *M. tubercilosis* Rv2361 (DecPP synthase)
E. coli: *E. coli* UPP synthase
A. thaliana: *A. thaliana* DedolPP synthase
Rer2p: *S. cerevisiae* Rer2p (DedolPP synthase)
HDS: *H. sapiens* HDS (DedolPP synthase)
HRT1: *H. brasiliensis* HRT1 (cis-prenyltransferase in latex)
HRT2: *H. brasiliensis* HRT2 (cis-prenyltransferase in latex)

Comparison of Conserved Regions of *E*- and *Z*-Prenyl Diphosphate Synthases



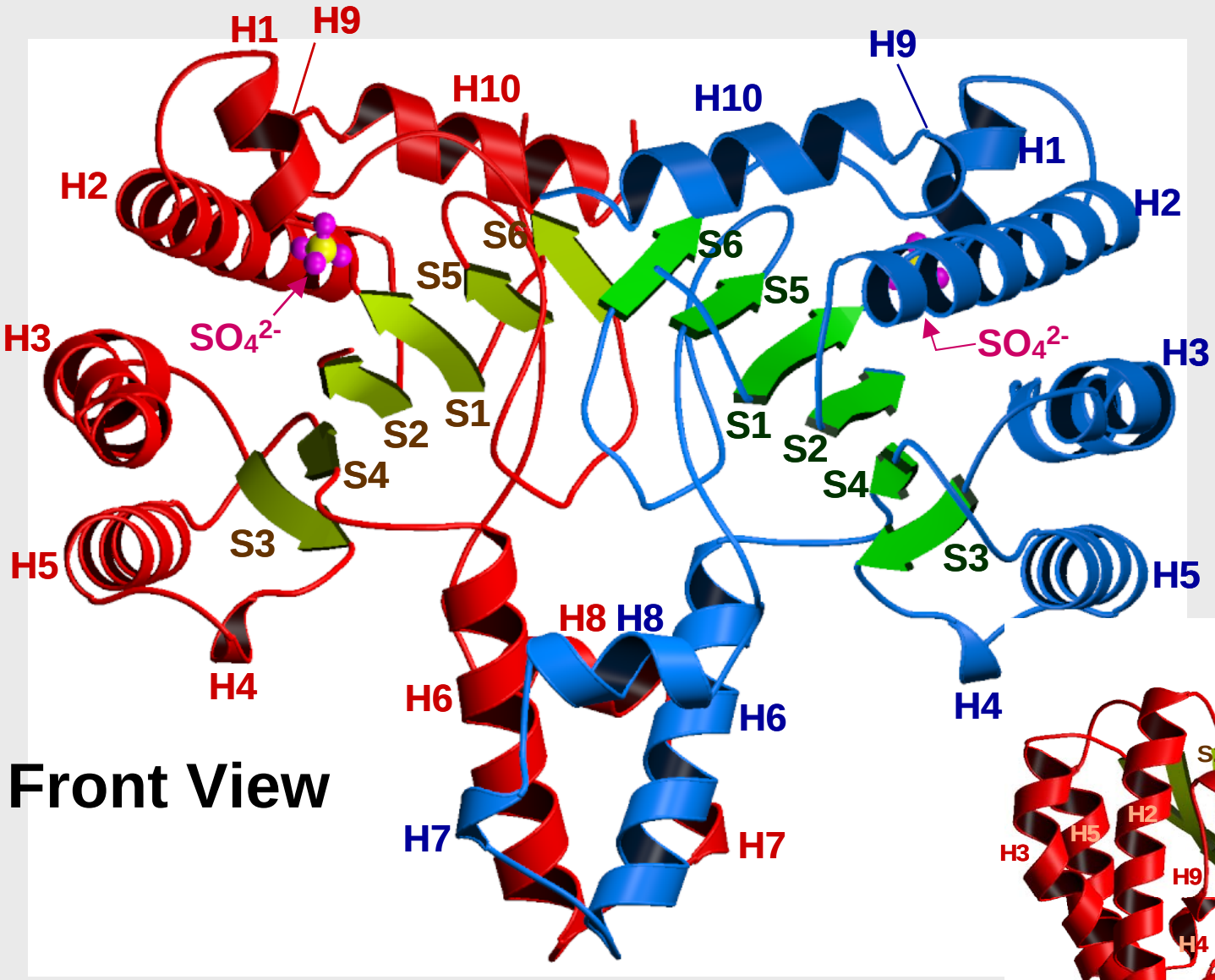
Crystal of UPS from *Micrococcus luteus* B-P 26

1.2 mm

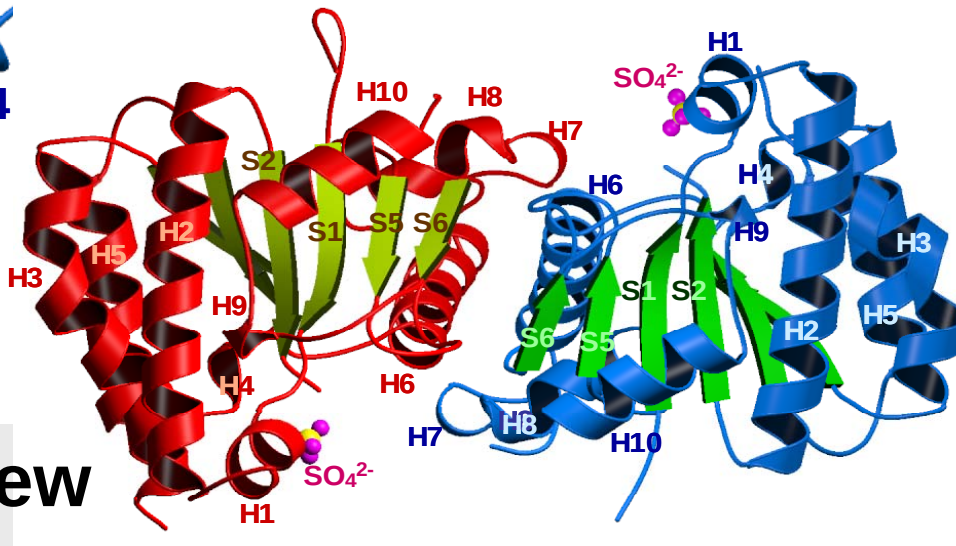
Resolution	2.2 Å
R_{merge}	3.2%
Completenes	83.7%

M. Fujihashi *et al.*, *Acta Crystallographica Section D*, 55 , 1606-1607 (1999)

Total Structure of *Micrococcus luteus* B-P 26 UPS



Front View

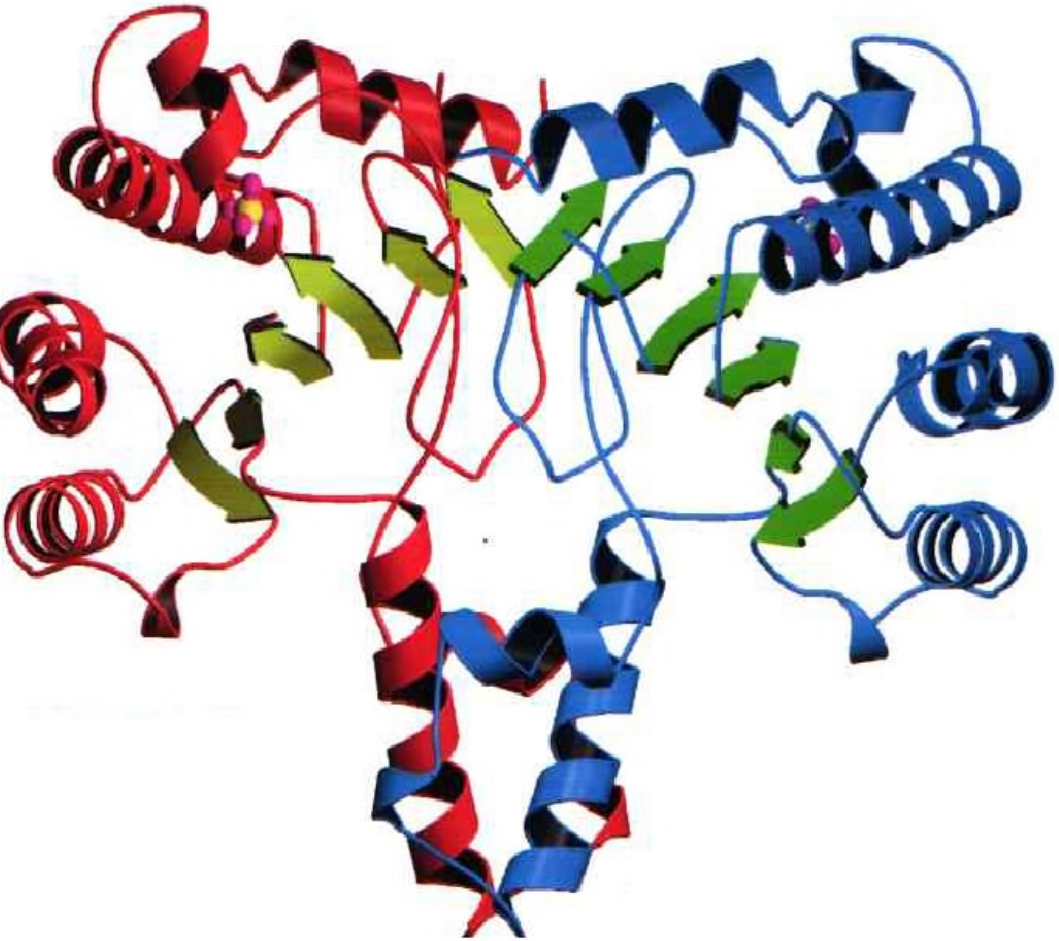


Top View

Fujihashi *et al.* Proc. Natl. Acad. Sci. USA (2001) 433

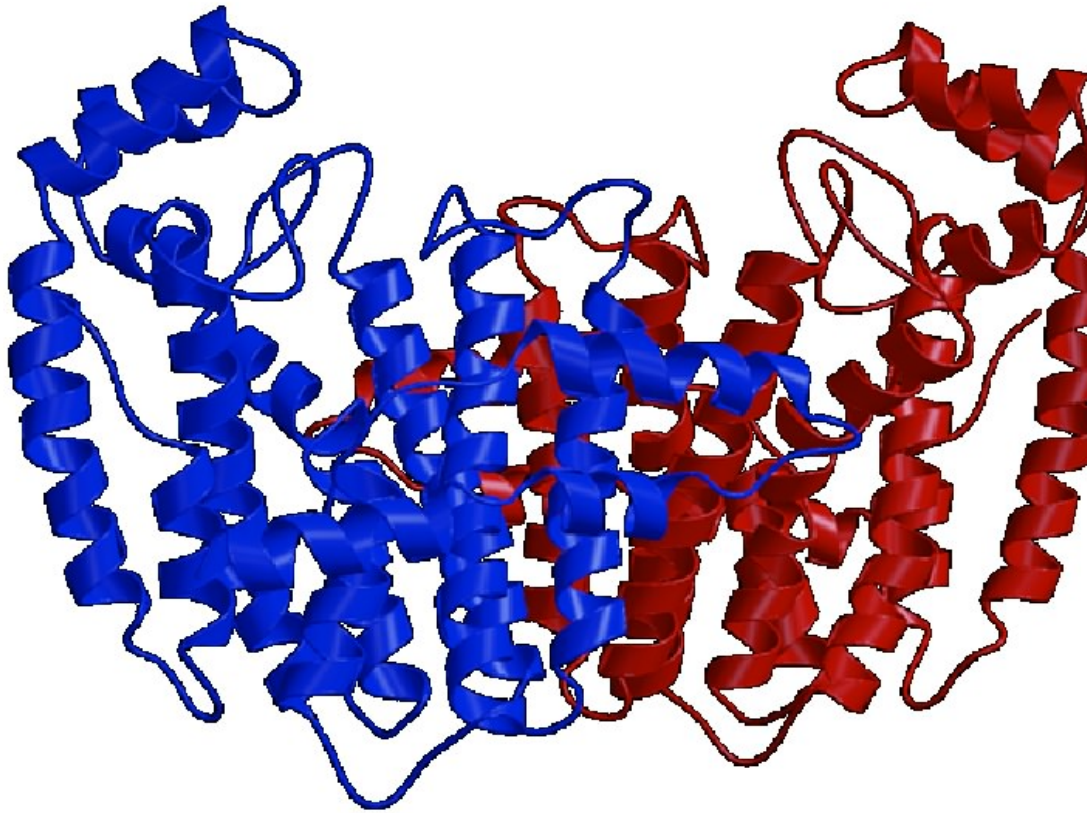
Crystal structure of *Micrococcus luteus* B-P 26 undecaprenyl diphosphate synthase and Avian FPP synthase

Micrococcus luteus B-P 26 UPS



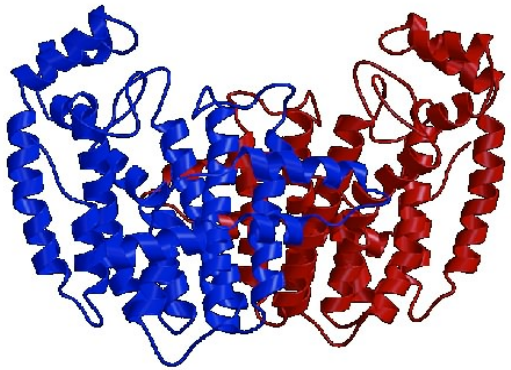
Fujihashi *et al.* Proc. Natl. Acad. Sci. USA (2001) 4337-42

Avian Liver FPP Synthase

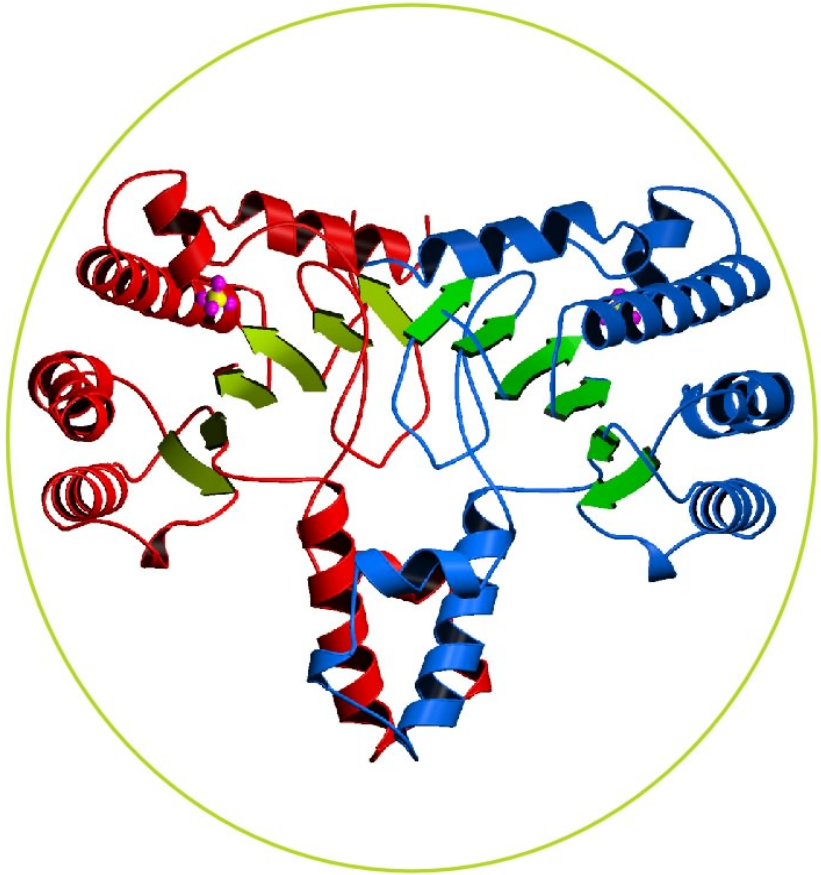


Tarshis, L. C. *et al.*, *Biochemistry*, **33**, 10871-10877 (19

The Structure of UPS is much different from the "Isoprenoid Synthase Fold"

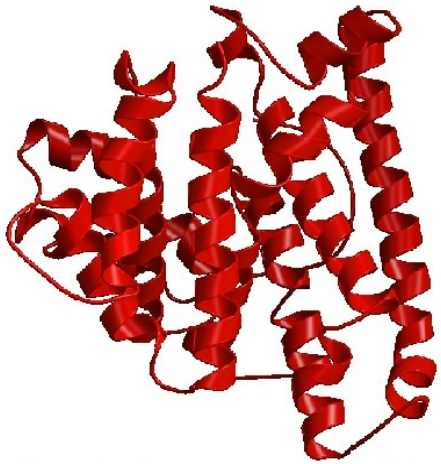


Farnesyl Diphosphate Synthase



UPS

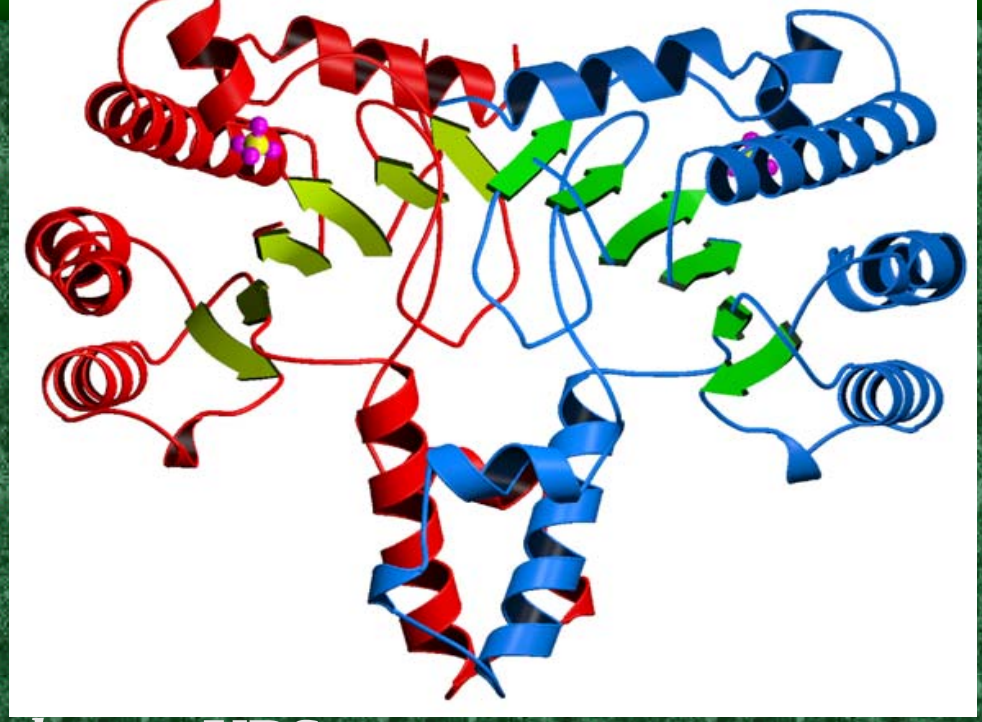
5-Epi-Aristolochene Synthase



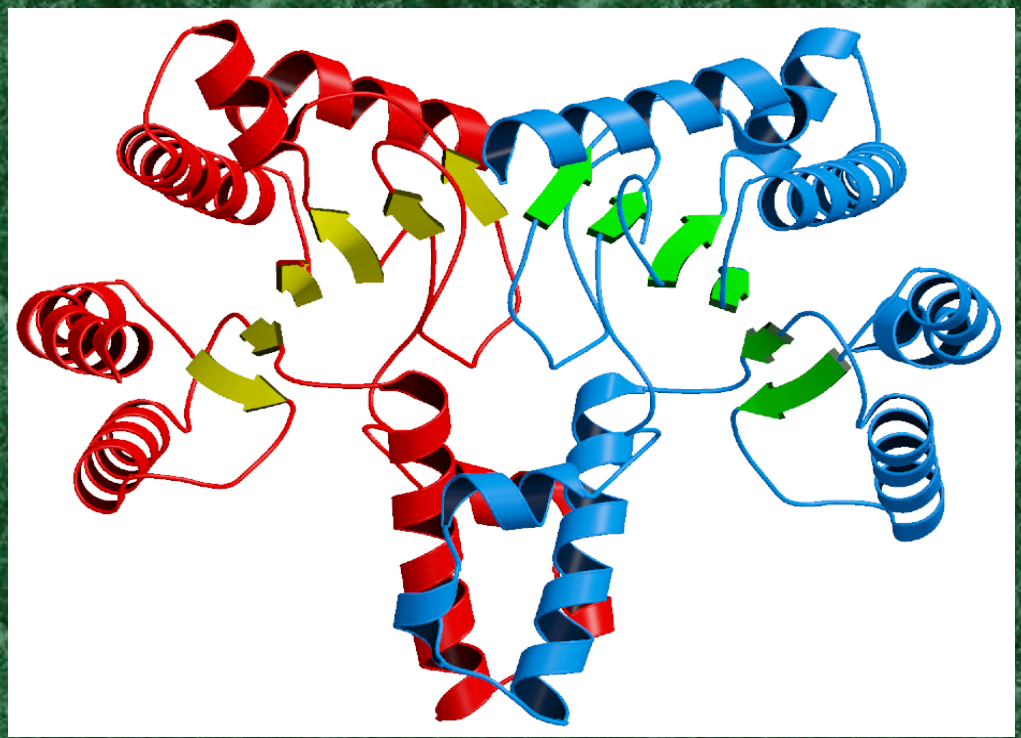
Pentalenene Synthase

Squalene Cyclase

Sacchettini and Poulter (1997) *Science* 277, 1788-1789.

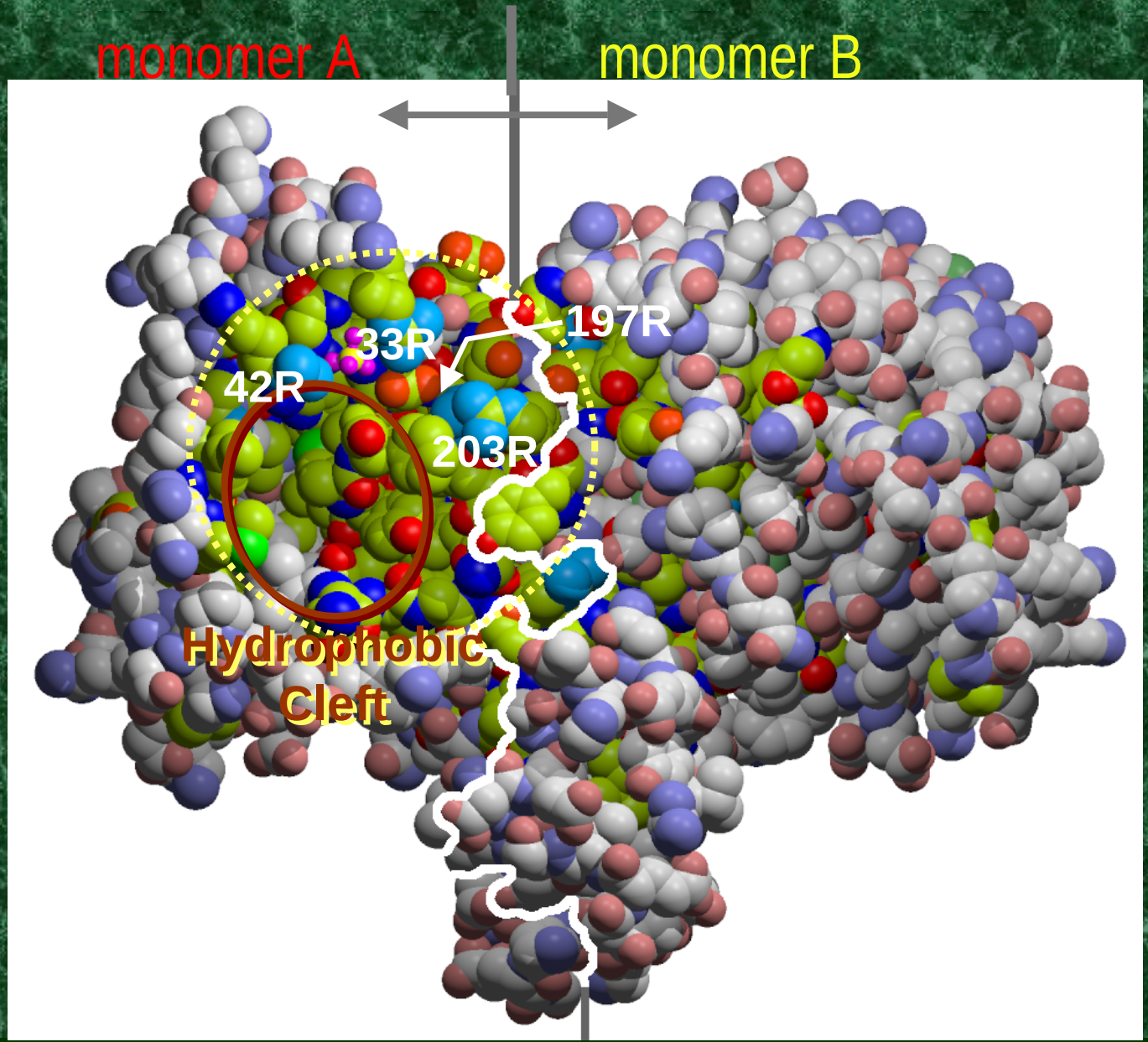


***M. luteus* UPS** [Fujihashi *et al*., *Proc. Natl. Acad. Sci.,USA.*, **98**, 4337-4342 (2001)]

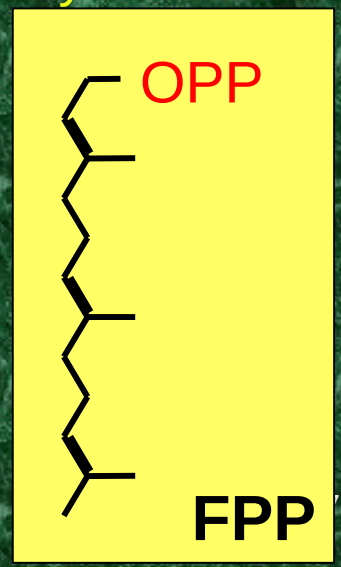


***E. coli* UPS** [Kaptan *et al*., *J. Biol. Chem.*, **276**, 47474-47482 (2001)]

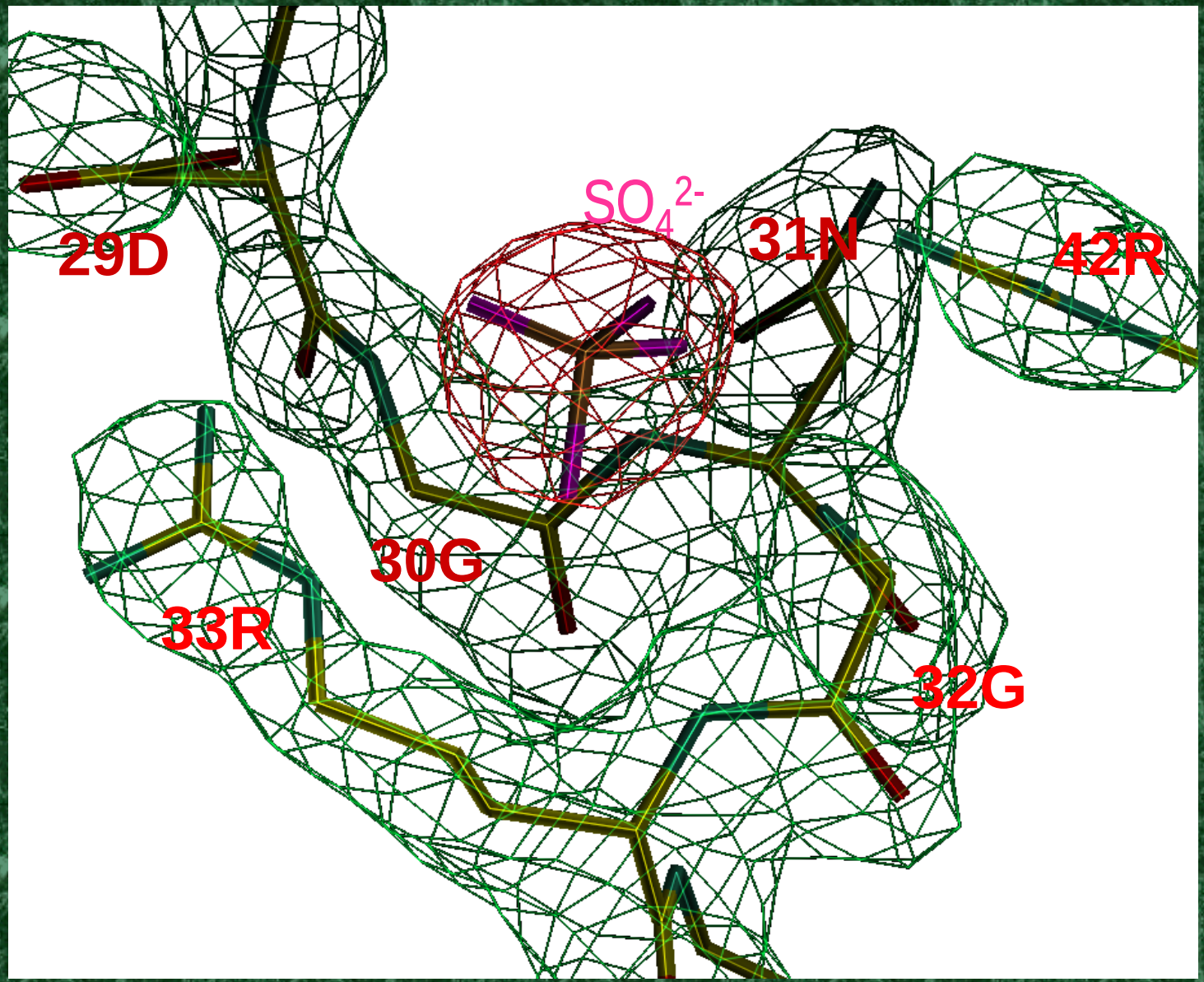
Conserved Regions



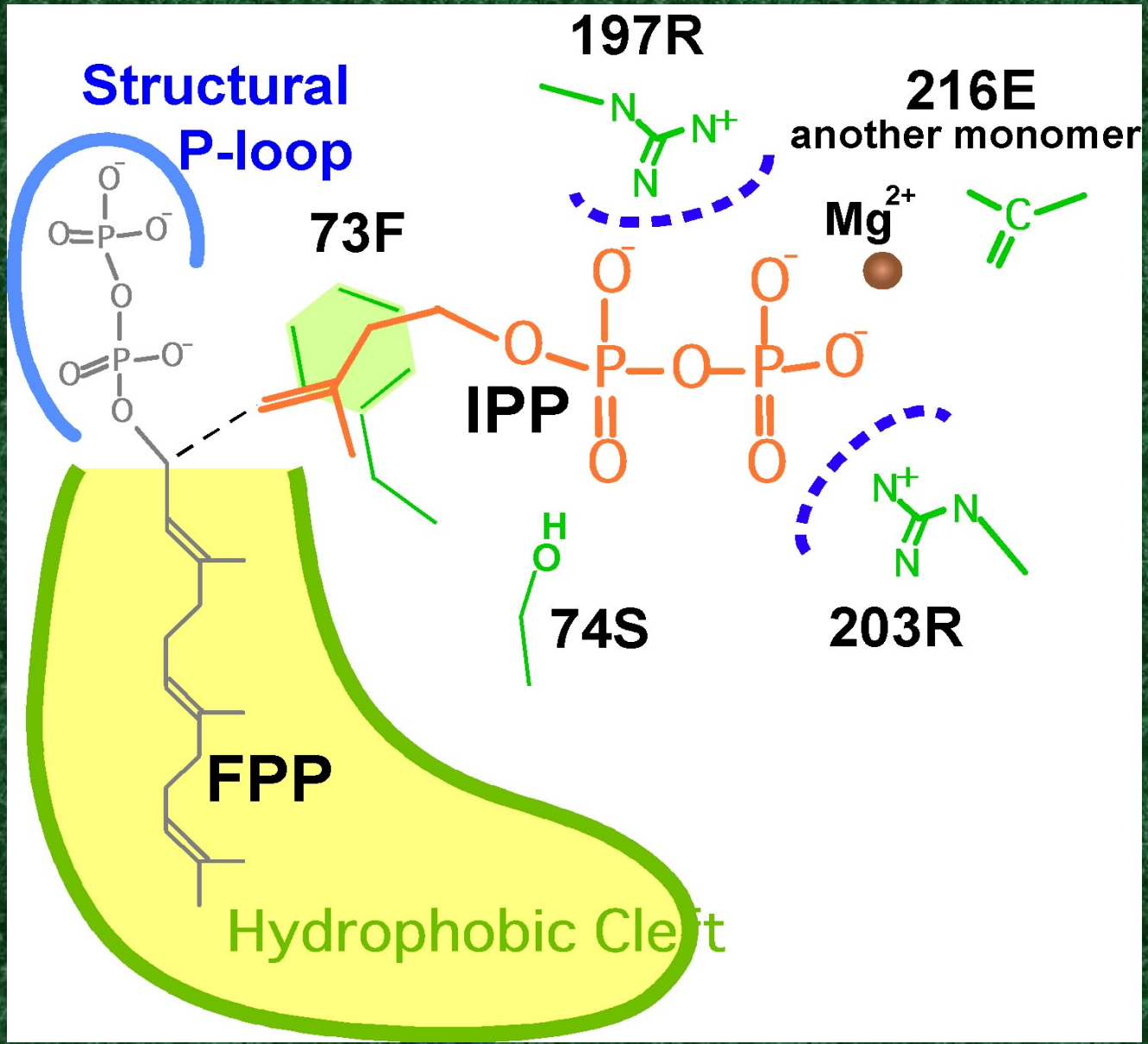
Allylic Substrate



A Sulfate Ion binds to the Structural P-loop

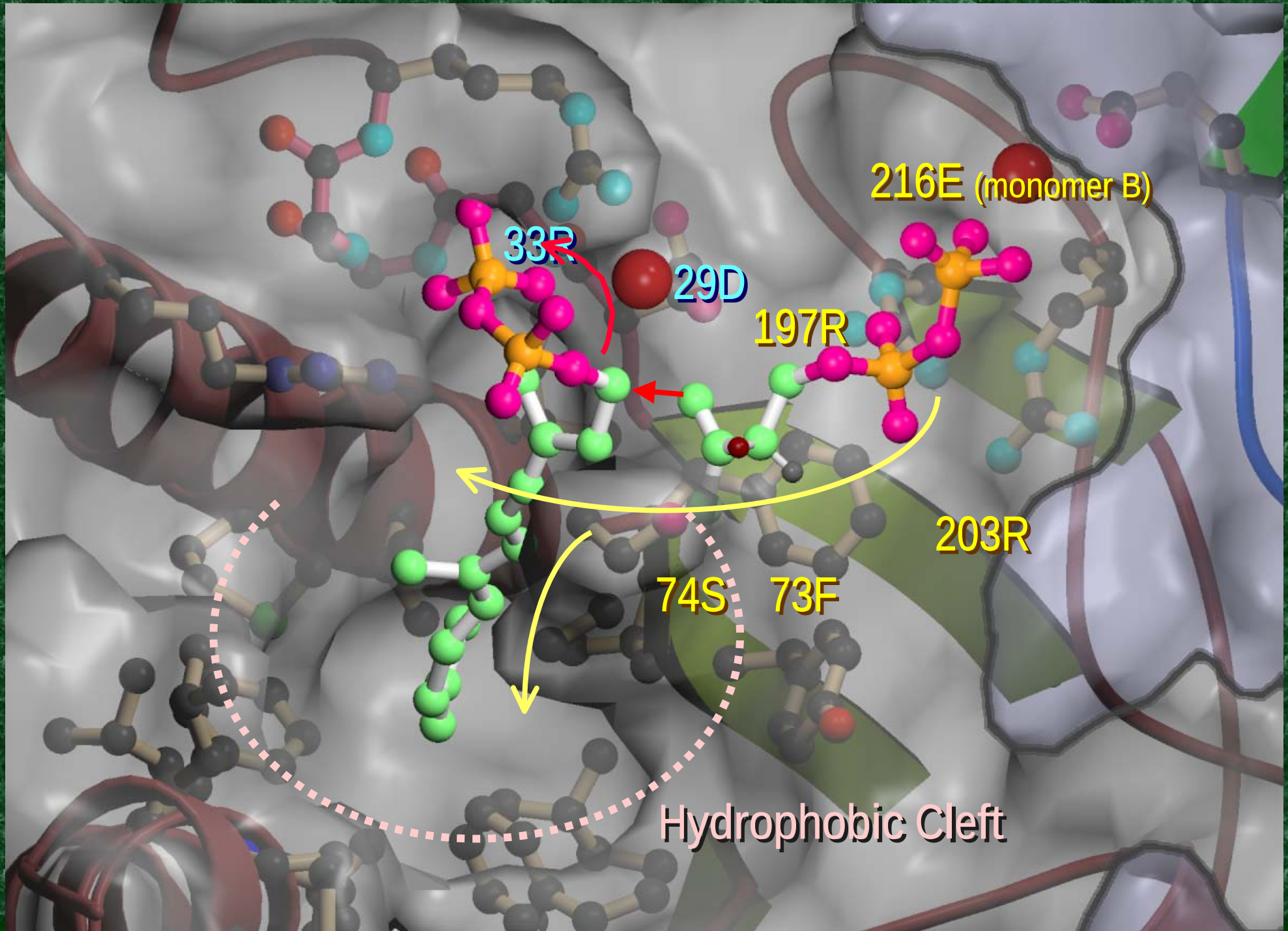


Proposed Substrate Binding Model of the UPP Synthase



- Kharel, Y., *et al., J. Biol. Chem.*, **276**, 28459-28464 (2001).

A Probable Catalytic Reaction Model

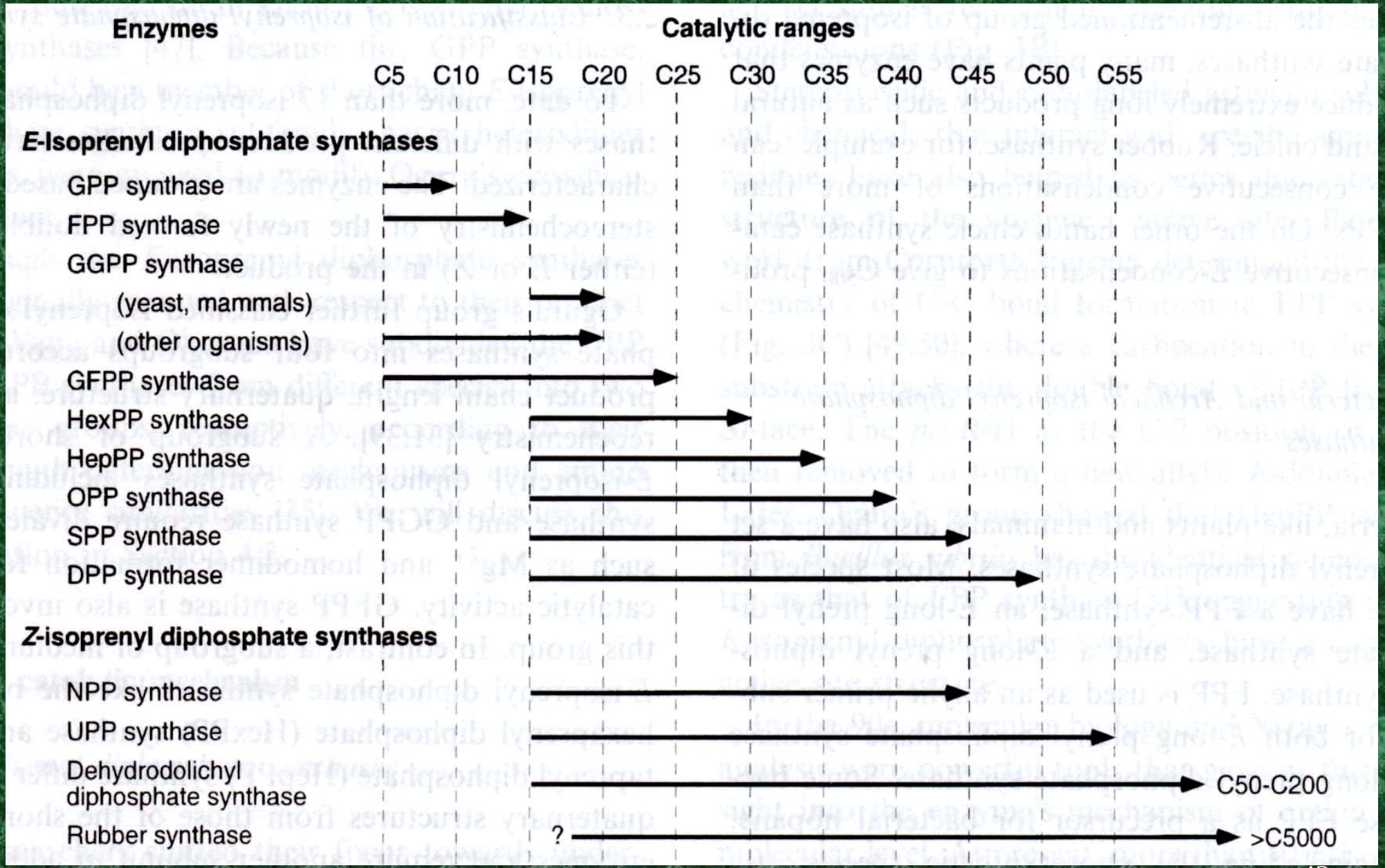


Chain Length Determination Mechanisms

1. *E*-Prenyltransferases

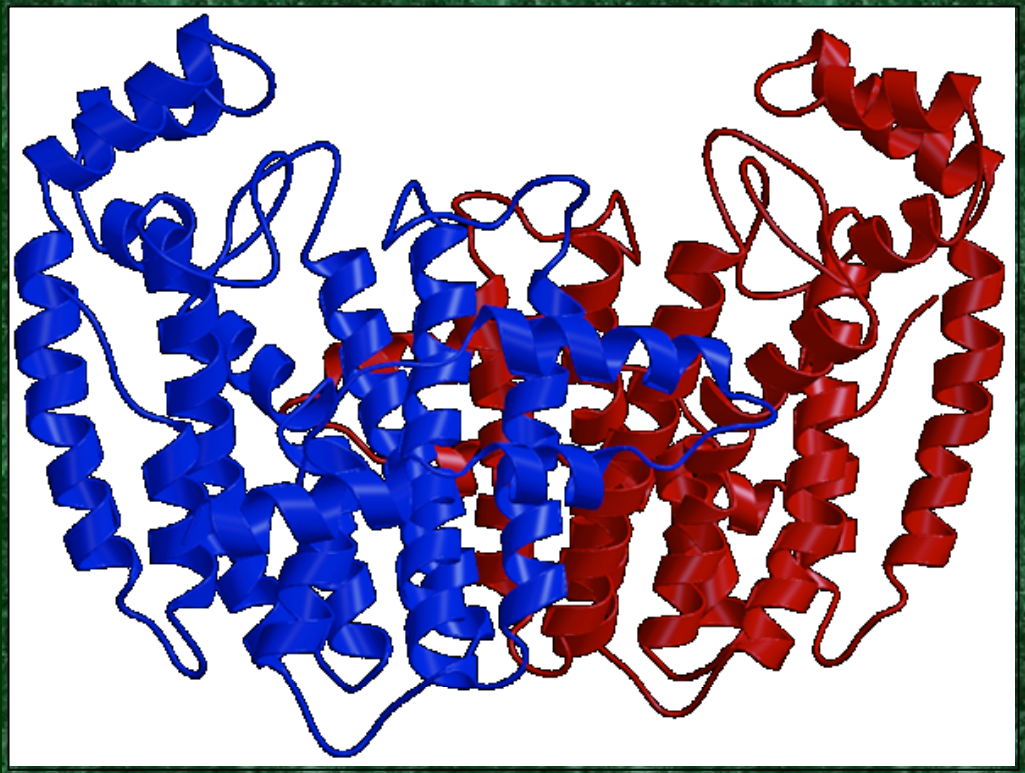
2. *Z*-Prenyltransferases

Catalytic Ranges of Prenyl Chain Elongation by Prenyltransferases so far Characterized



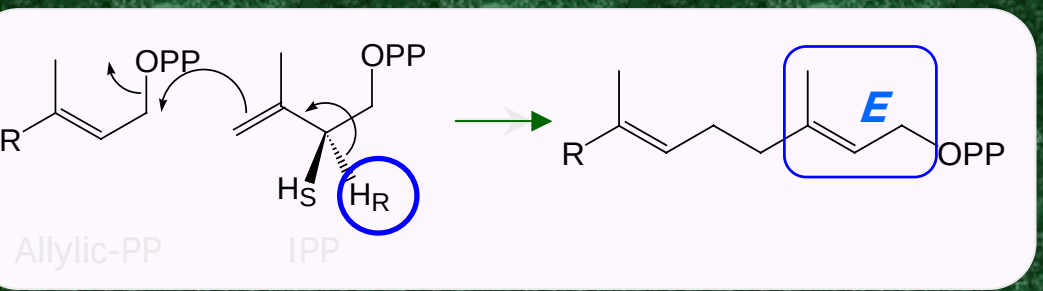
Prenyltransferases

- *trans*-prenyltransferase

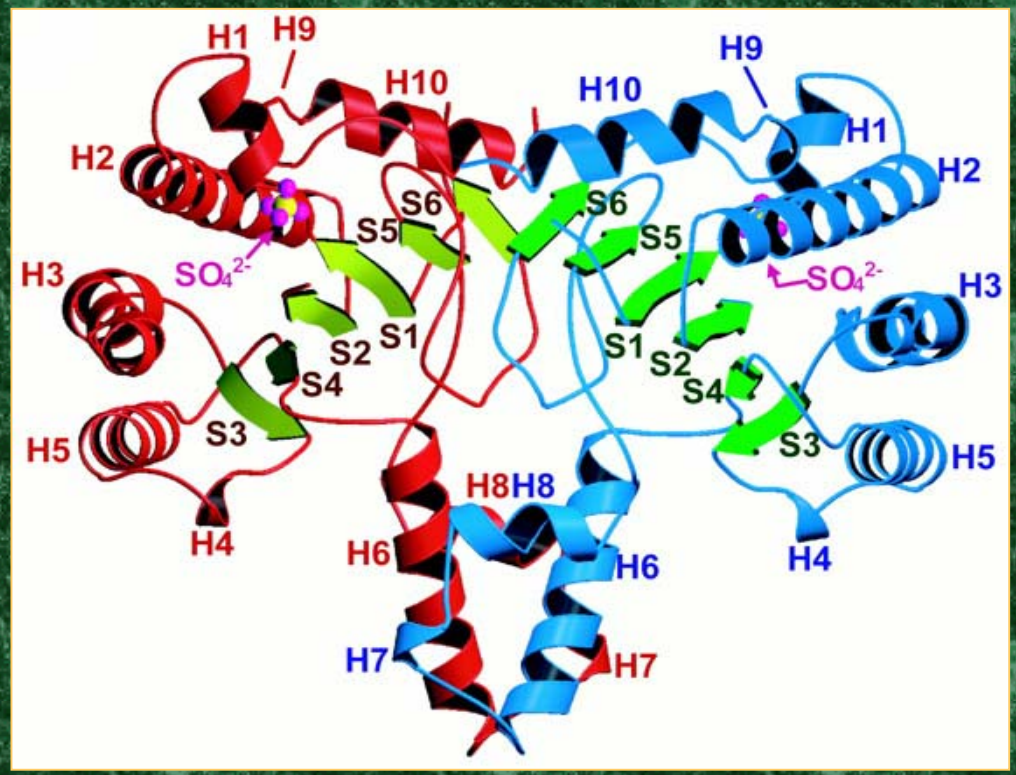


Avian Liver FPP synthase

Tarshis, L. C. *et al. Biochemistry*, 33, 10871-10877 (1994)

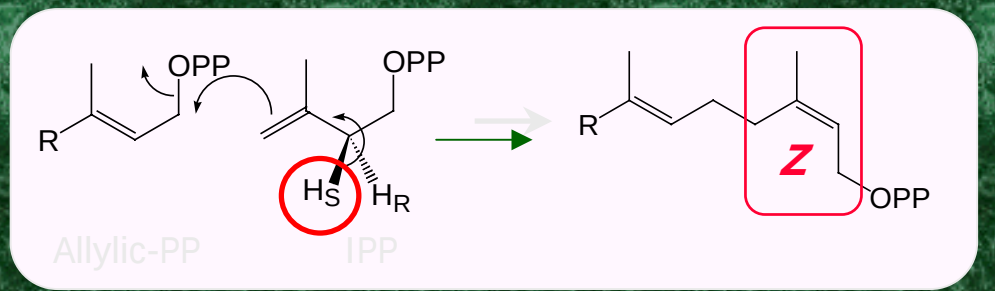


- *cis*-prenyltransferase

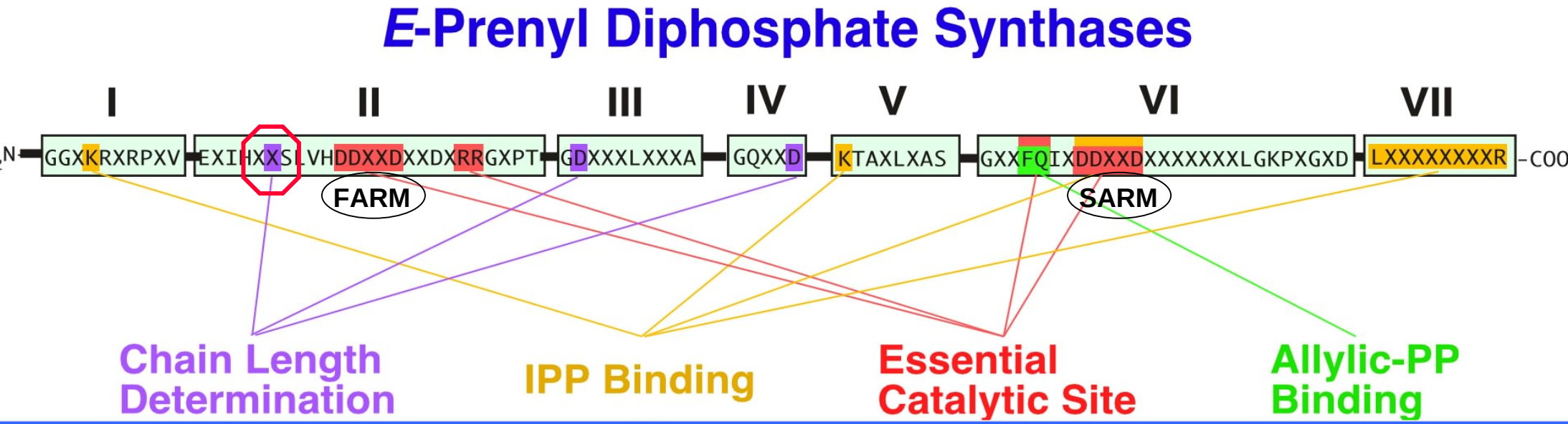


***Micrococcus luteus* B-P 26 UPP synthase**

Fujihashi *et al. Proc. Natl. Acad. Sci.* 4337-42 (2001)

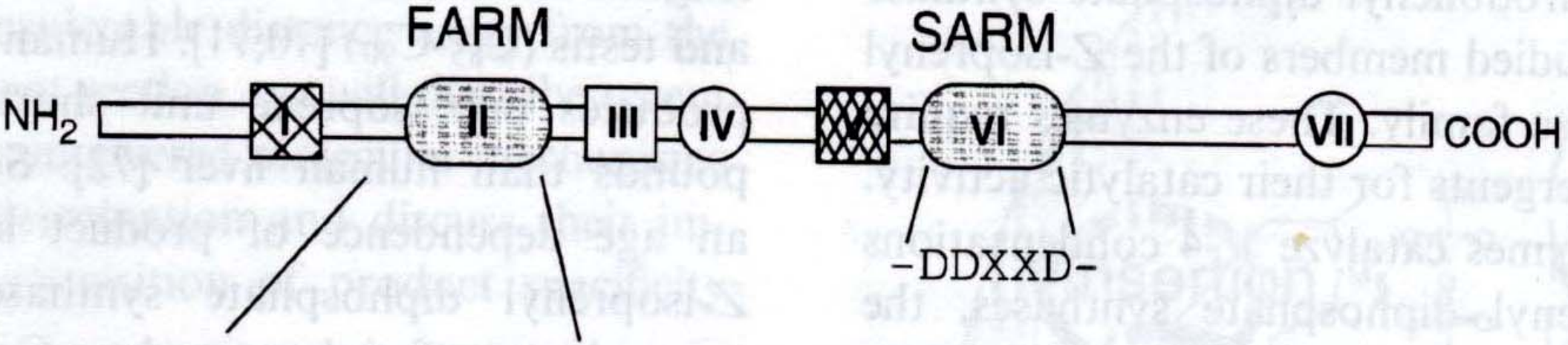


Roles of the Conserved Regions of *trans*-Prenyl Chain Elongating Enzymes



Koyama, T. et al., Biochemistry, 35, 9533-9538 (1996); Koyama, T. et al., Biochem. Biophys. Res. Commun., 212, 681-686 (1995).; Koyama, T. et al., Can. J. Chem., 72, 75-79 (1994); Koyama, T. et al., Biochemistry, 33, 12644-12648 (1994); Ohnuma, S. et al., J. Biol. Chem., 271, 18831-18837 (1997); Ohnuma, S. et al., J. Biol. Chem., 272, 5192-5198 (1997)

First Aspartate-Rich Motif Second Aspartate-Rich Motif

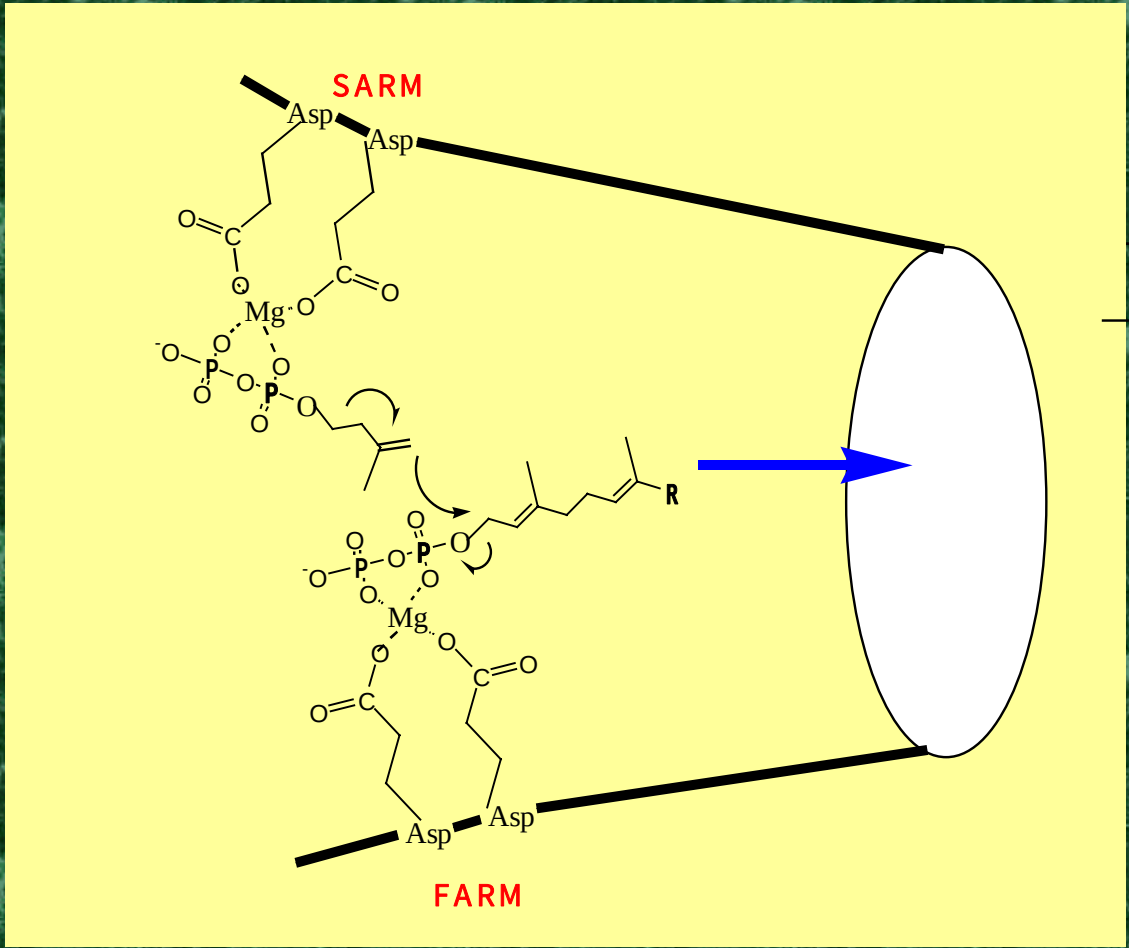


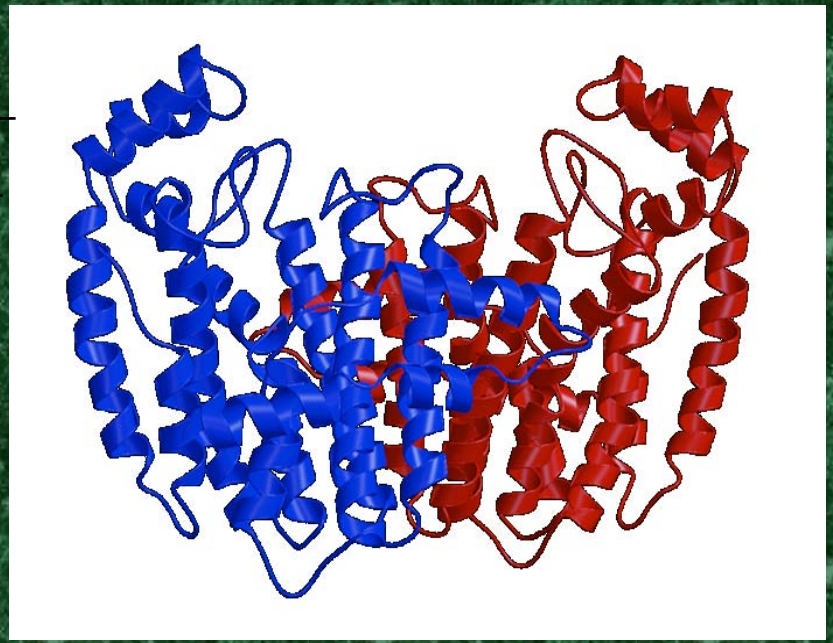
159-	TMSLMHDDLPCMD-	GPP syn, Peppermint
91-	AFFLVADDI--MD	FPP syn, Avian
77-	AYSLIHDDLPAMD	FPP syn, *Escherichia coli*
75-	TFTLVHDDI--MD	GGPP syn, *Sulfolobus acidocaldarius*
119-	AASLILDDLPCMD	GGPP syn, *Arabidopsis thaliana*
87-	AASLILDDMPCMD	GGPP syn, *Erwinia uredovora*
58-	NASLLIDDI--ED	GGPP syn, Human
186-	TASLLHDDV--ID	HexPP syn, *Saccharomyces cerevisiae*

CLD region

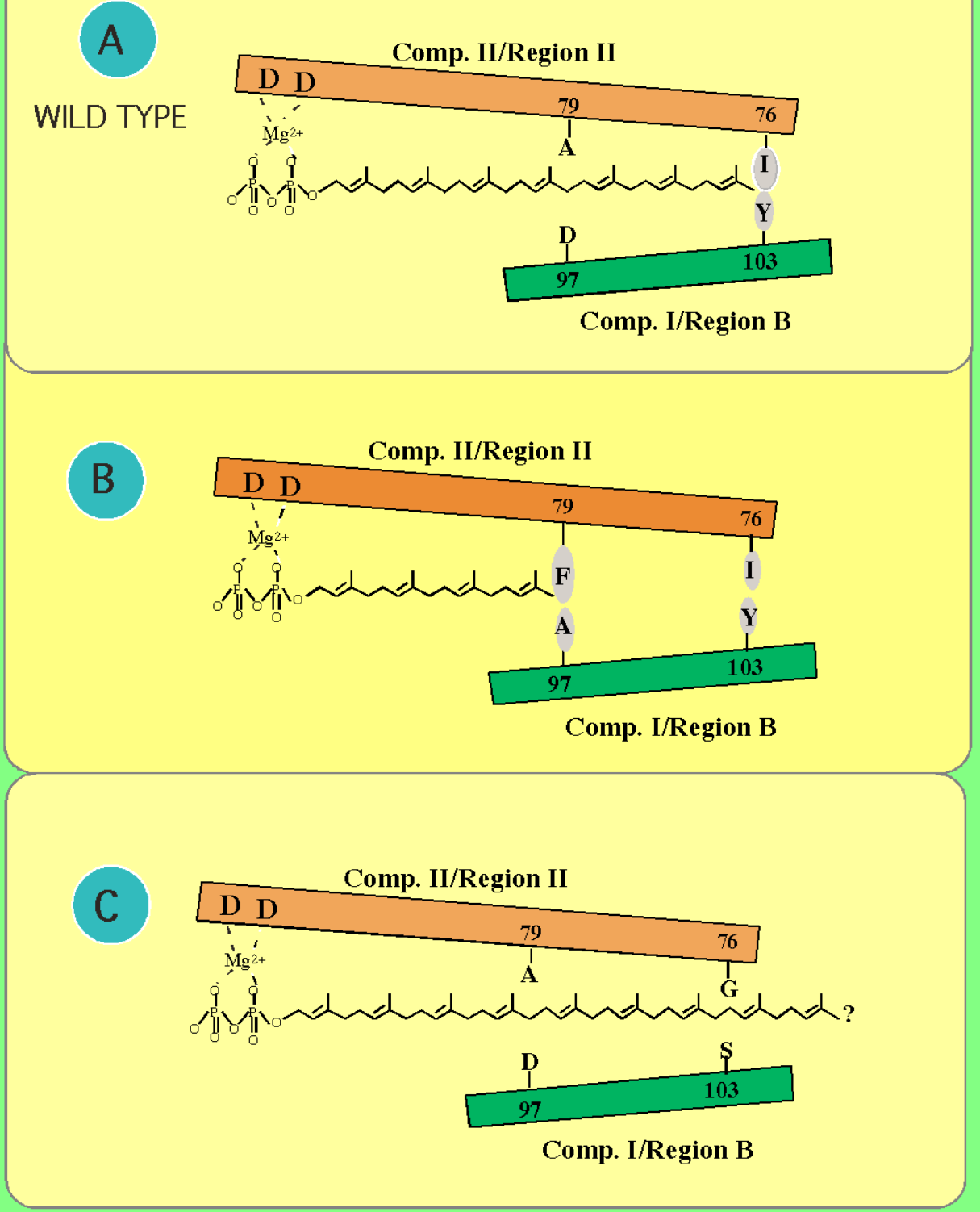
Wang and Ohnuma, TIBS 1999, BBA 2000

Product Chain Length Determination Mechanism of *trans*-Prenyltransferase



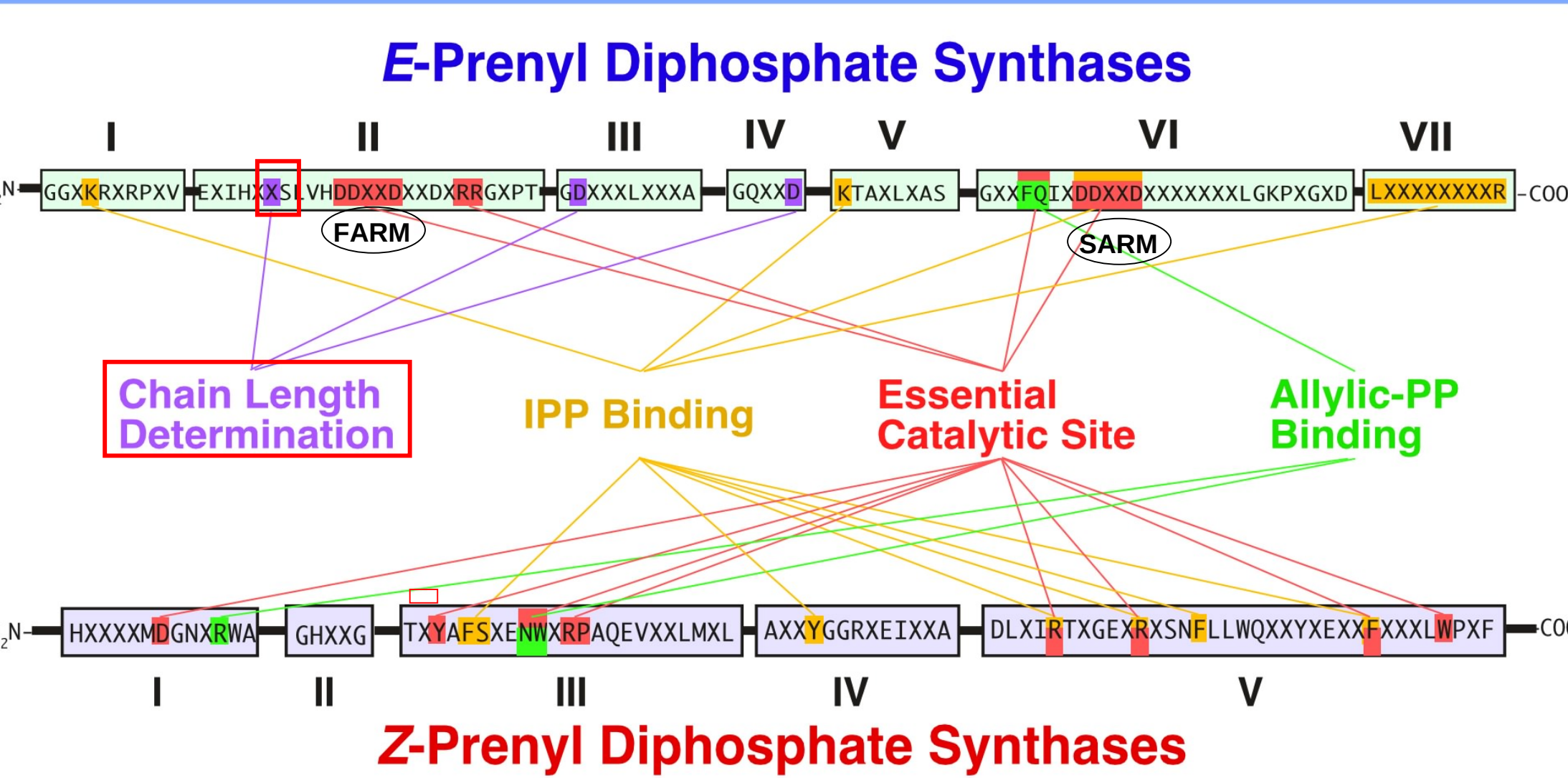


S. Ohnuma *et al., J. Biol. Chem.*, 271, 10087-10095 (1996).;
L. C. Tarshis *et al., Proc. Natl. Acad. Sci., USA*, 93, 15018-15023 (1996).;
S. Ohnuma *et al., J. Biol. Chem.*, 271, 30748-30754 (1996).

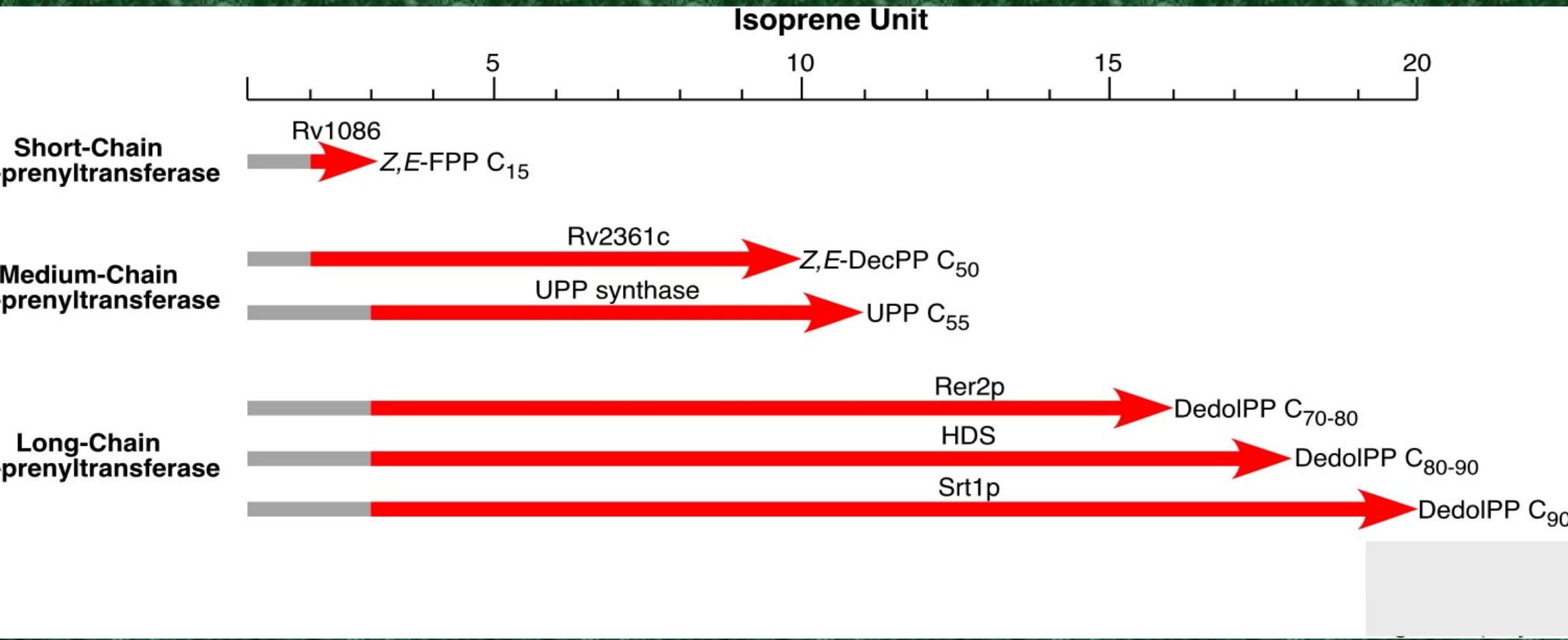


K. Hirooka *et al. Eur. J. Biochem.*, **267**, 4520-4528 (2000).

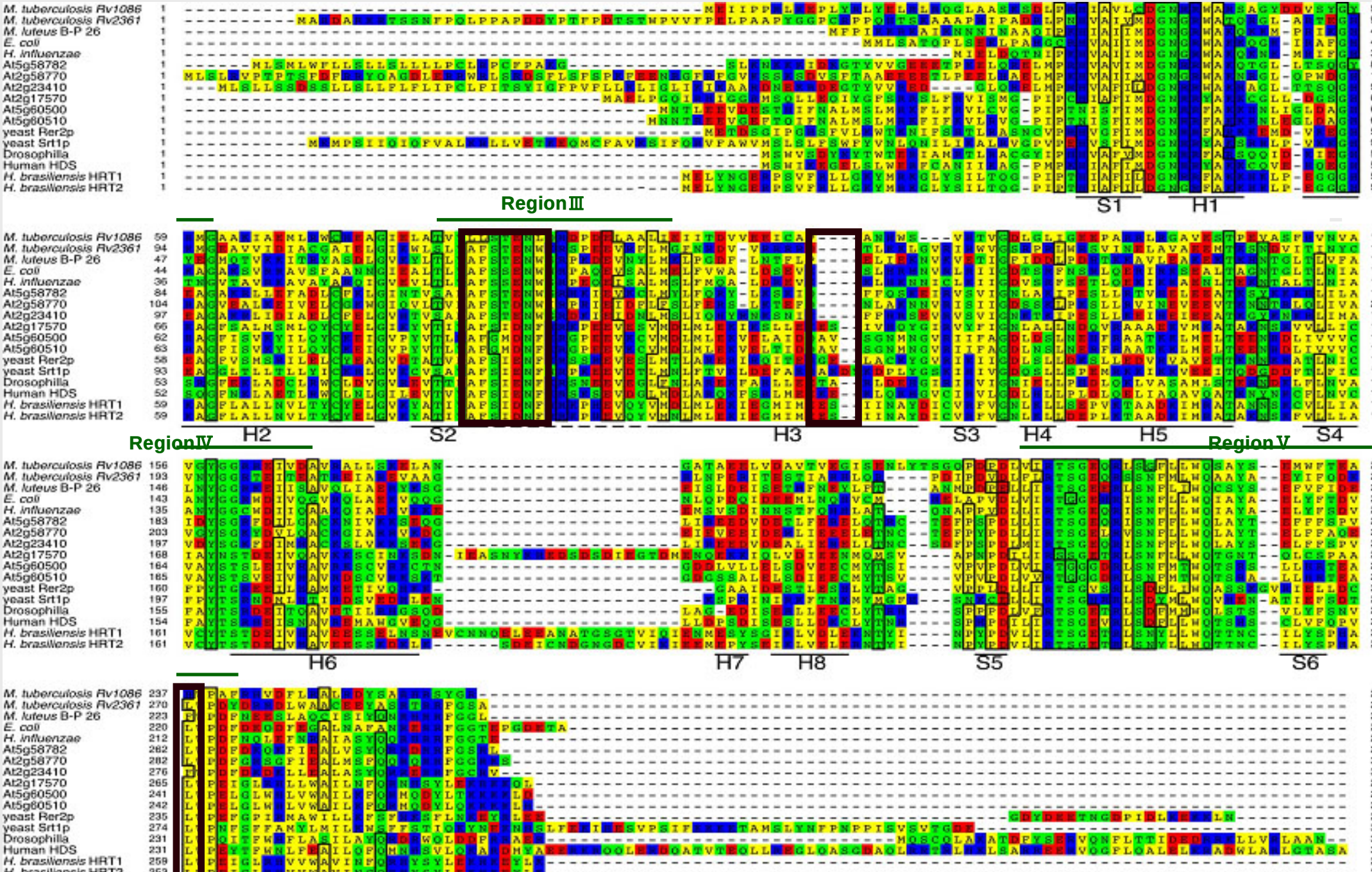
Comparison of Conserved Regions of *E*- and *Z*-Prenyl Diphosphate Synthases



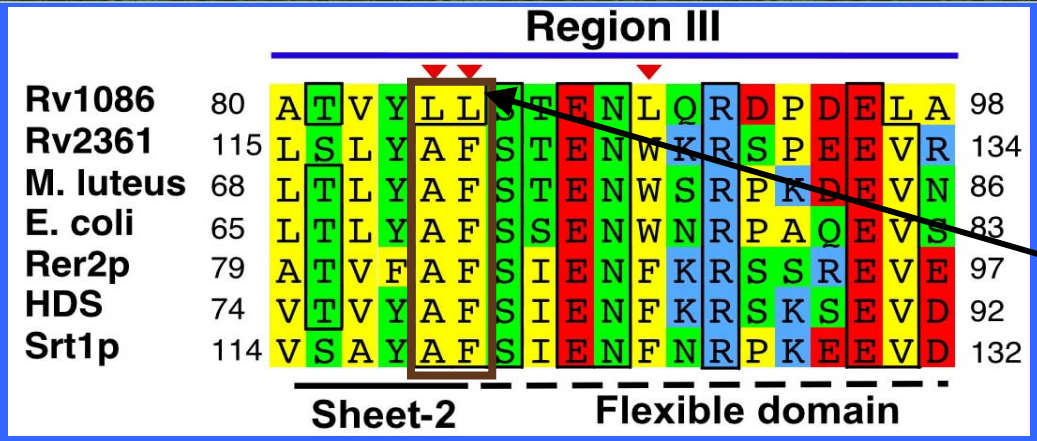
Classification of *cis*–Prenyltransferases

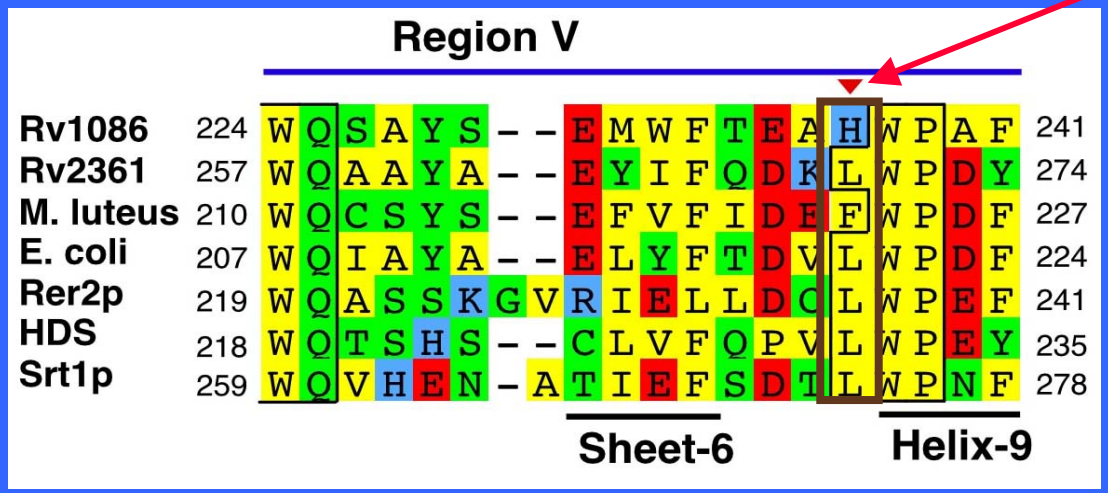


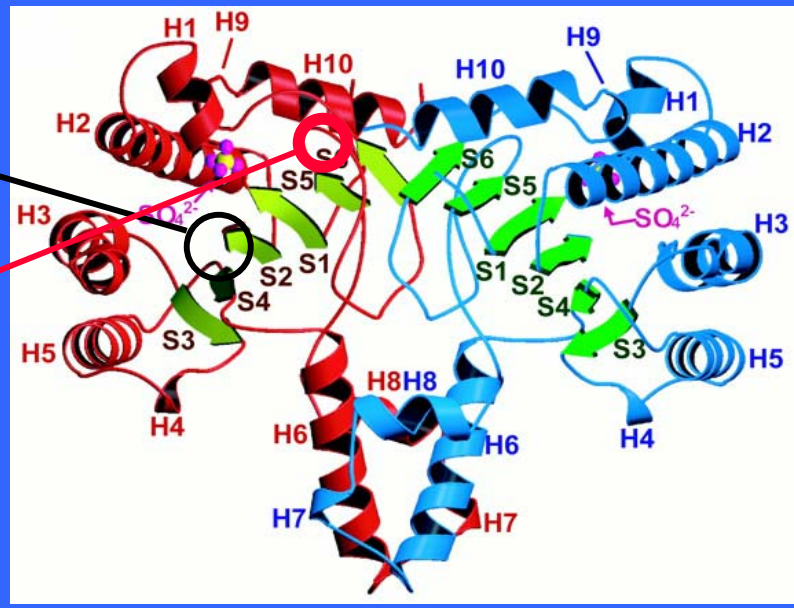
Amino Acid Alignments of *cis*-Prenyltransferases



Characteristic Regions in Short *cis*-Prenyltransferase







M. Luteus B-P 26 UPP synthase
Fujihashi *et al. Proc. Natl. Acad. Sci.* 4337-42 (2001)

- ***cis*-FPS WT** — **82- VYLLSTENLQR –92** — **235- EAHWP -239**
- **AF (L84A / L85F)** — **82- LYAFSTENLQR –92**
- **L84A** — **82- LYALSTENLQR –92**
- **L85F** — **82- LYLFSTENLQR –92**
- **H237L** — **235- EALWP -239**

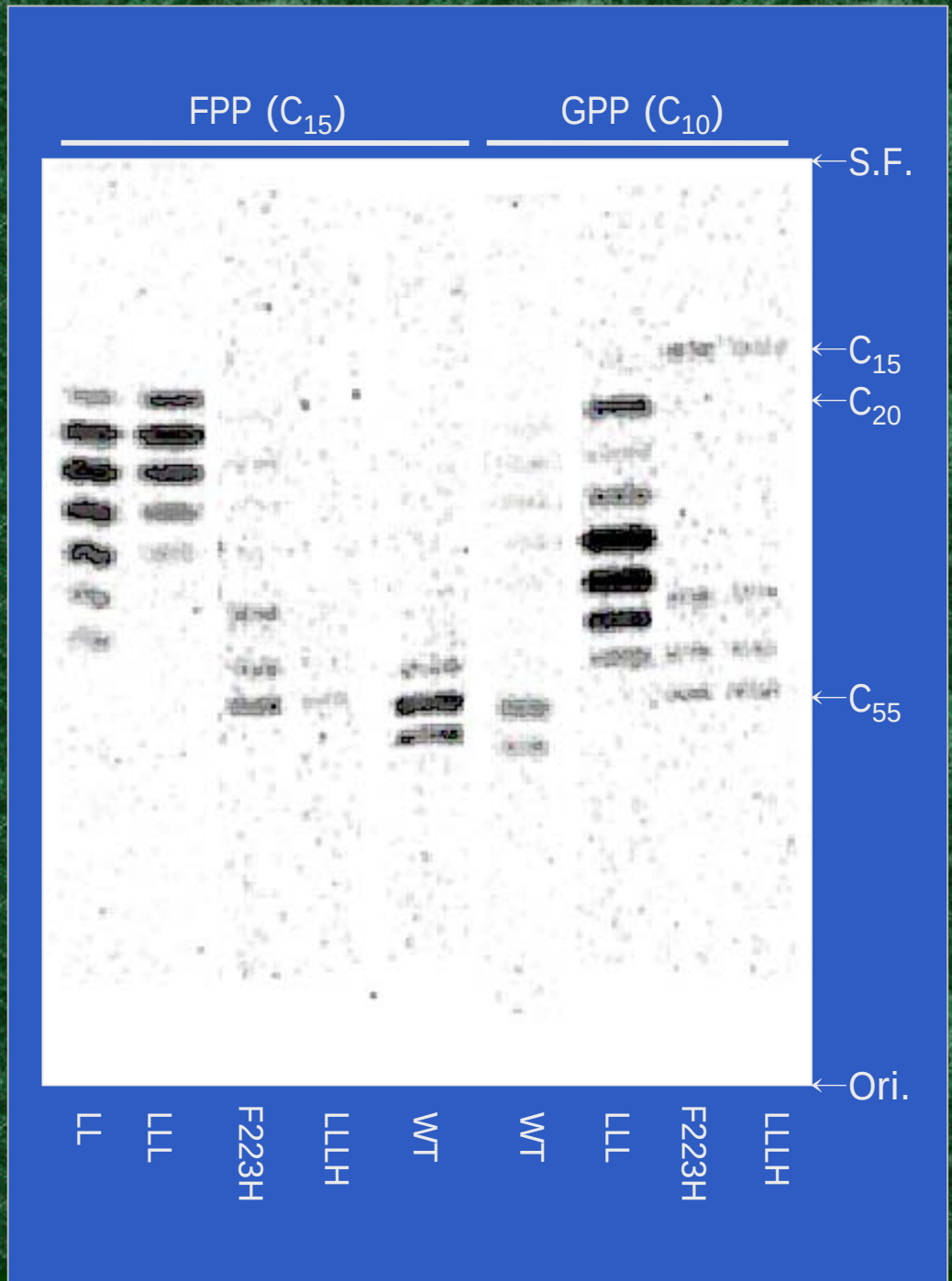
Product Analysis of UPS Mutant Enzymes

***M. luteus* UPS mutant**

Reversed phase TLC

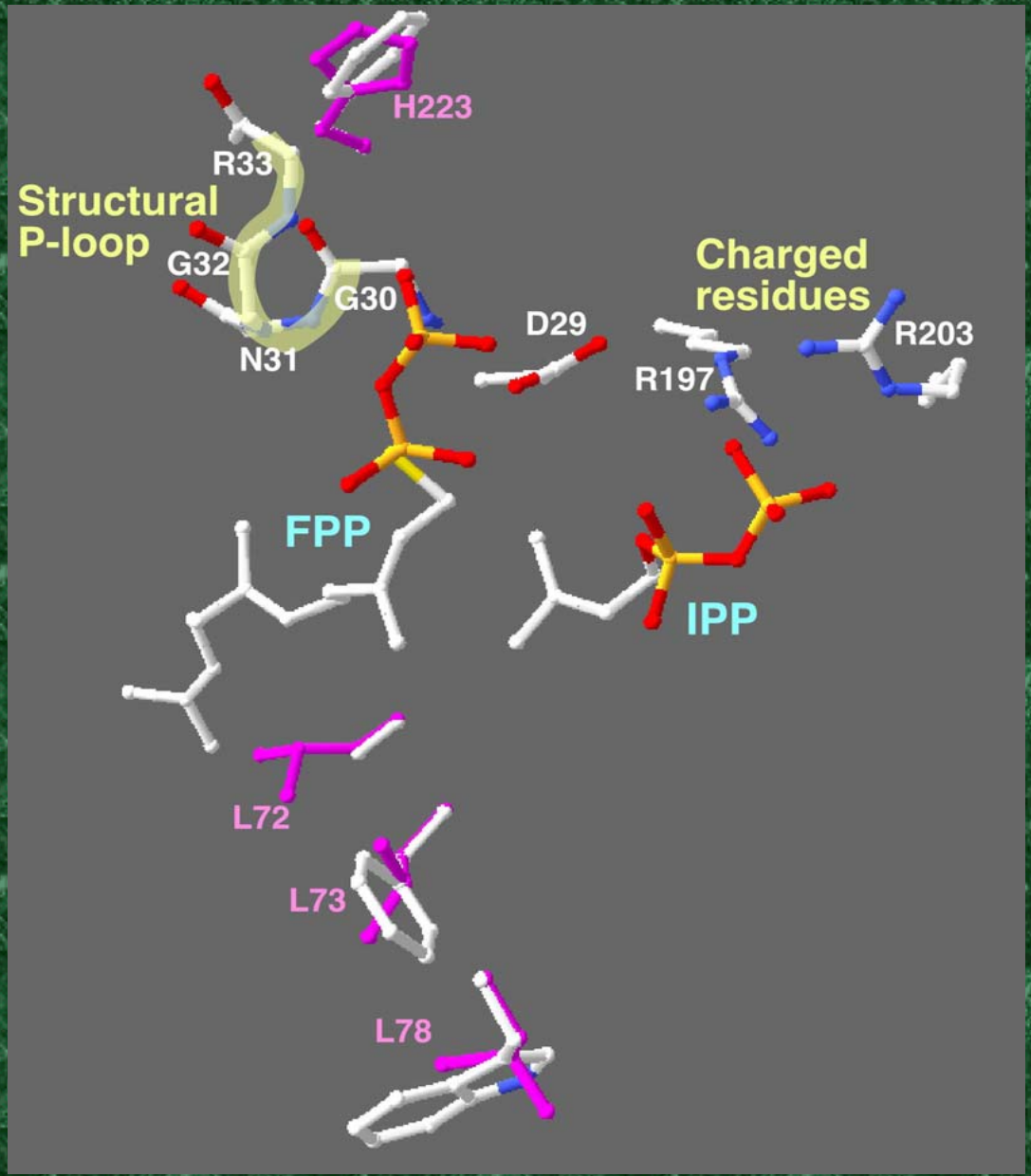
solvent···Acetone/water(19:1)

• UPS WT	70- LY**AF**STEN**W**SR –80	221- DE**F**WP -225
• LL	70- LY**LL**STENWSR –80	
• LLL	70- LY**LL**STEN**L**SR –80	
• F223H		221- DE**H**WP –225
• LLLH	70- LY**LL**STEN**L**SR –80	221- DE**H**WP -225



Kharel, Y. et al., *FEBS J.*, 273, 647-657 (2006)

Structure Model of the LLLH Mutant of UPS



- Leu-84

Ala-72 in *M. luteus* UPS

···close to the ω-end carbon of FPP

⇒Side-chain of Leu may **interfere** with elongation of product.

Chang, S.Y. *et al.*, ***Protein Sci.***, 13, 971 (2004)

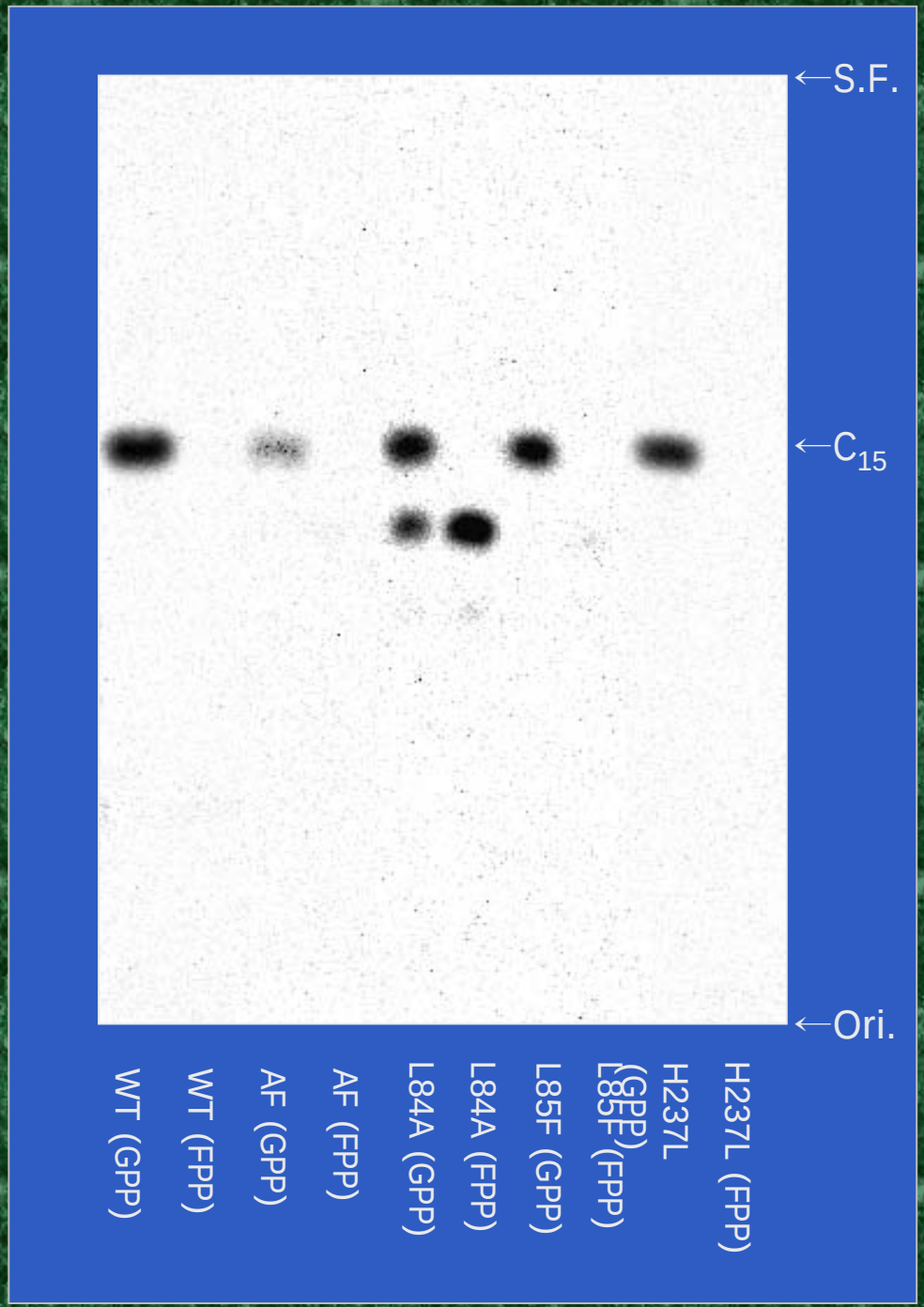
Product Analysis of FPS Mutant Enzymes

***M. tuberculosis* FPS mutant**

Reversed phase TLC

solvent･･･MeOH/Acetone(8:2)

- *cis*-FPS WT 82- VY**LL**STENLQR –92 265- EA**H**WP -239
- AF 82- LY**AF**STENLQR –92
- L84A 82- LY**A**LSTENLQR –92
- L85F 82- LYL**F**STENLQR –92
- H237L 265- EA**L**WP -239

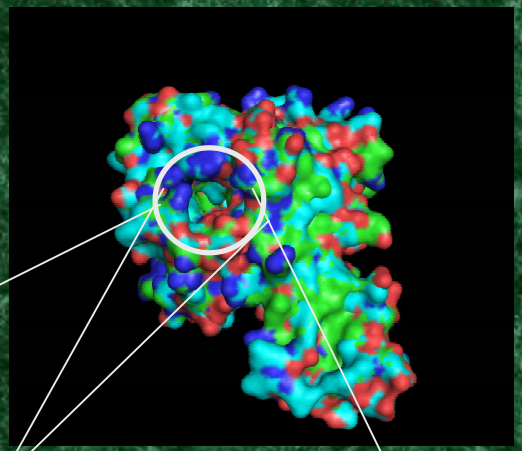


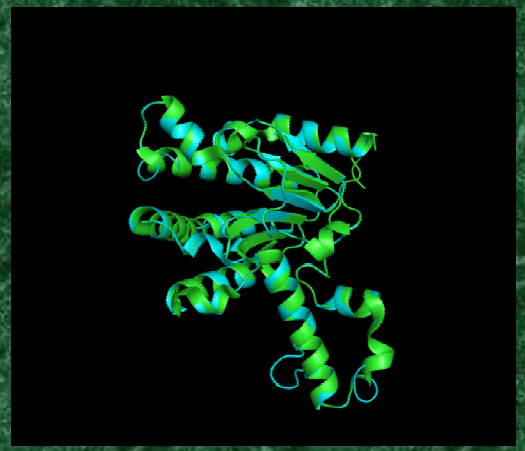
Noike, M. et al., *Biochem. Biophys. Res. Commun.*, 377, 17-22 (2008)

分子モデリング

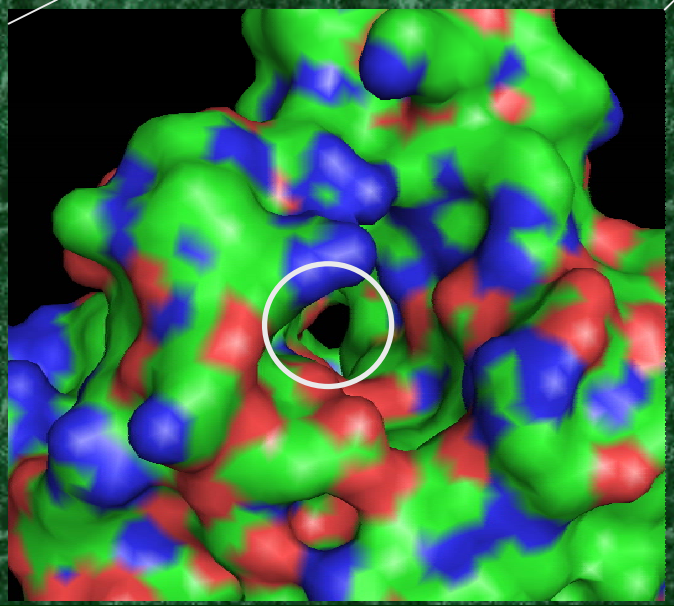
SWISS-MODEL programを使用し、
Thermobifida fusca cis-FPSの一次配列からモデルを作成

テンプレート：
E. coli UPS FPP結合型構造
（PDB ID : 1v7u）　Chang *et al.* (2004)

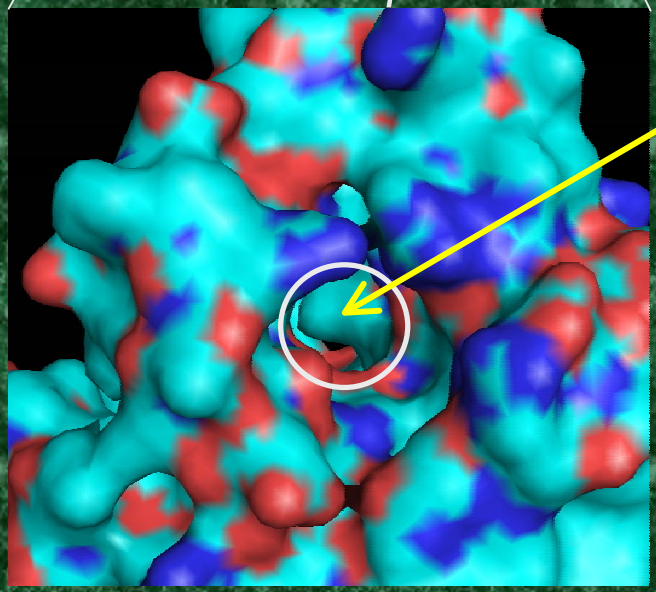




E. coli UPS



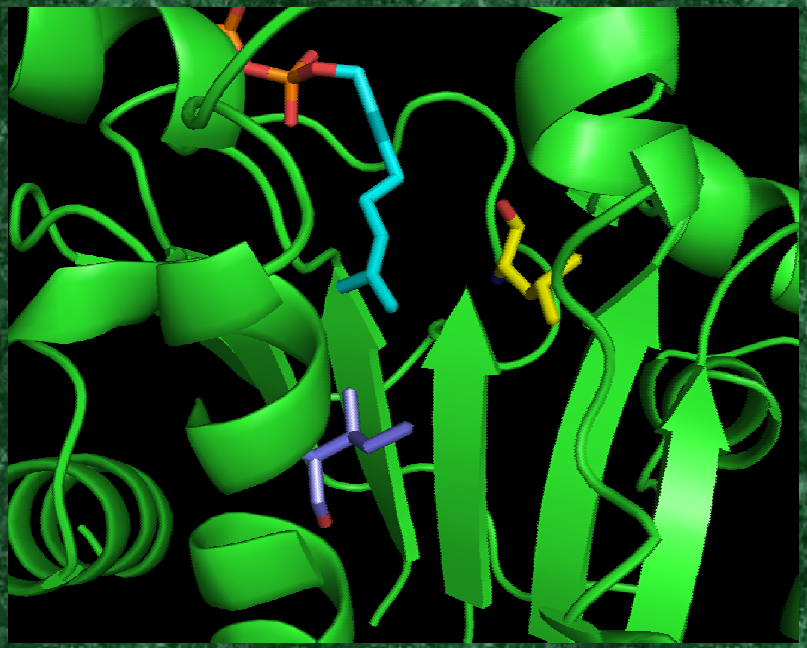
T.fusca FPS

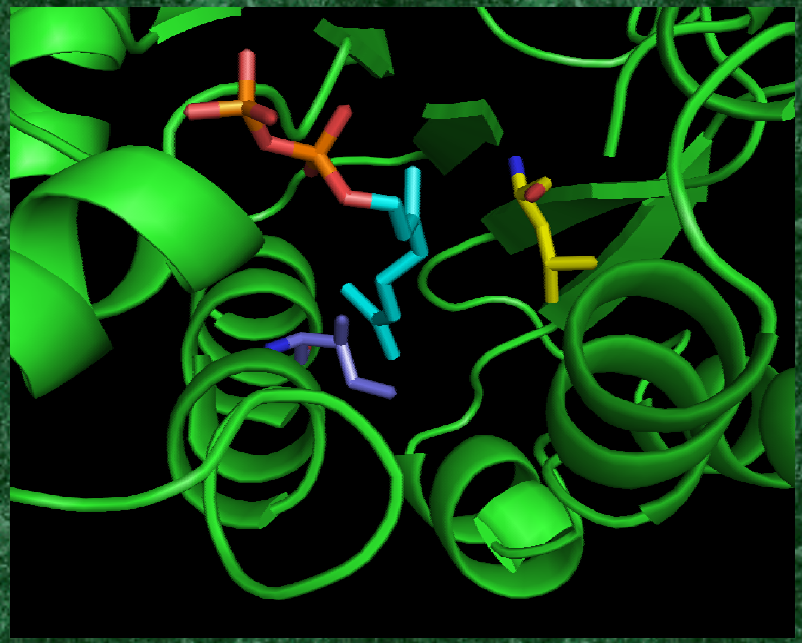


Ile-59

T. fusca cis-FPS の基質結合型モデル

T. fusca cis-FPSの基質結合型モデル





: GPP

: Ile59

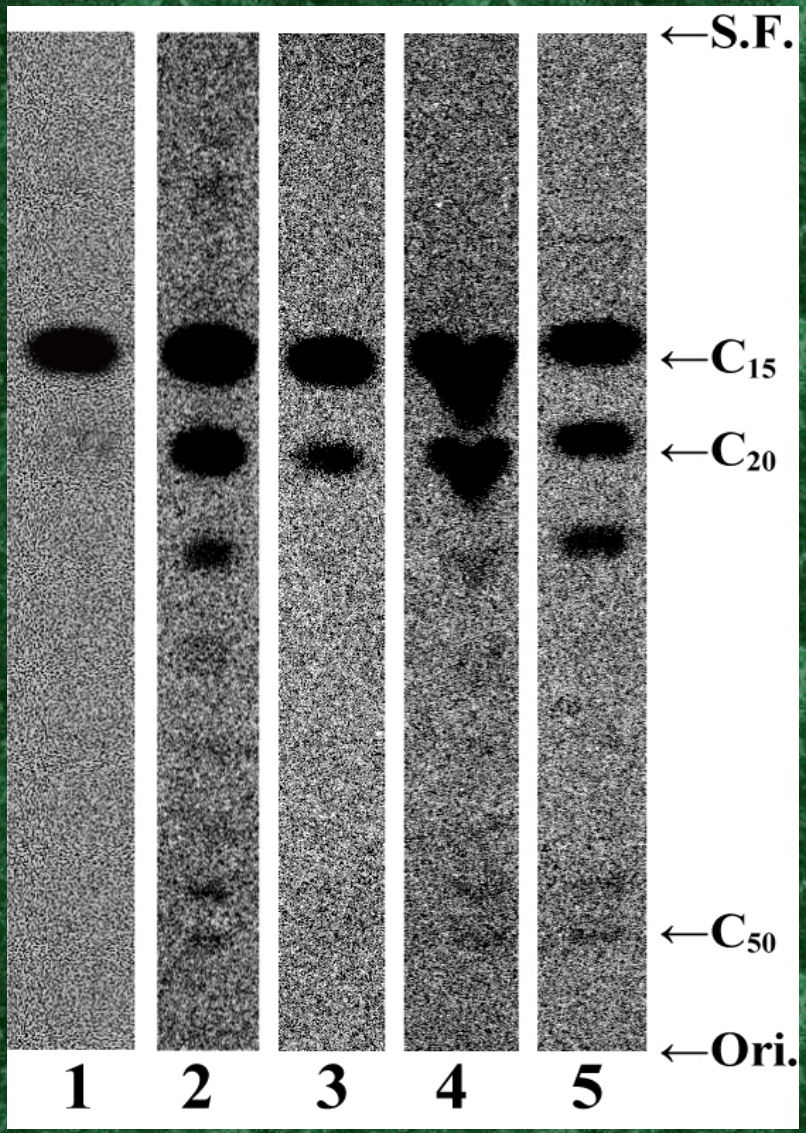
: Leu78

Ile残基の周辺の部分アライメント

Cg_FPS	55	G A K K **I** G E M V	63
Ce_FPS	103	G A K K **I** G E L V	111
Tf_FPS	55	G A E K **I** F E L L	63
Mt_FPS	61	G A A K **I** A E M L	69
Mt_DecPS	96	G E A V V **I** D I A	104
MI_UPS	49	G M Q T V K K I T	57
Ec_UPS	46	G A K S V R R A V	54
Hp_UPS	44	G V K T L K D I T	52
Hs_DedolPS	54	G F N K L A E T L	62
Sc_rer2	60	G F V S M S R I L	68
Sc_srt1	95	G G L T L L T L L	103

変異体の生成物鎖長解析

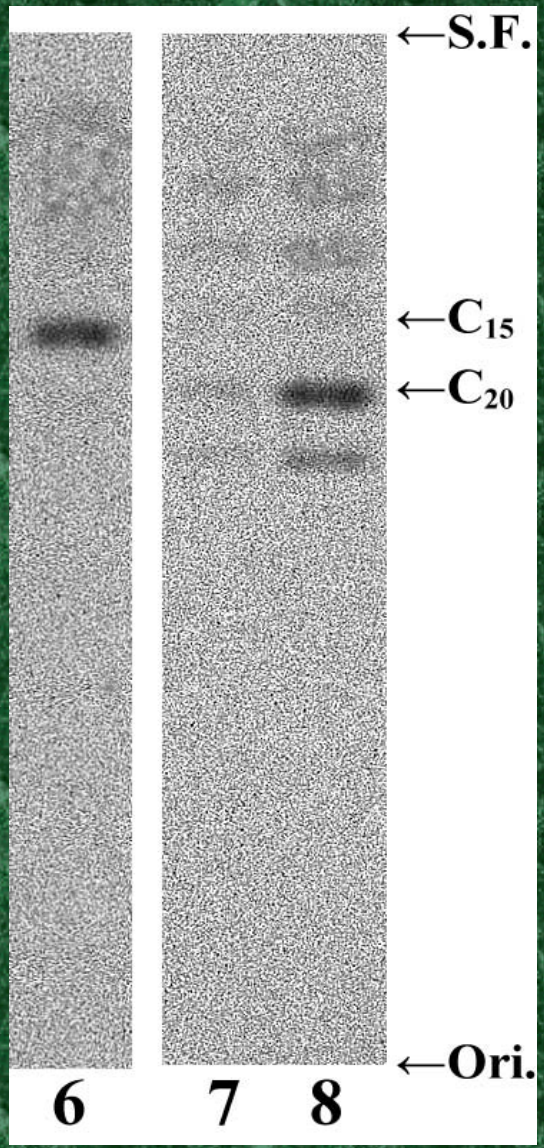
Ile59変異体



1：WT+GPP
2：I59V+GPP
3：I59L+GPP
4：I59M+GPP
5：I59A+GPP

展開溶媒
MeOH:Acetone=8:2

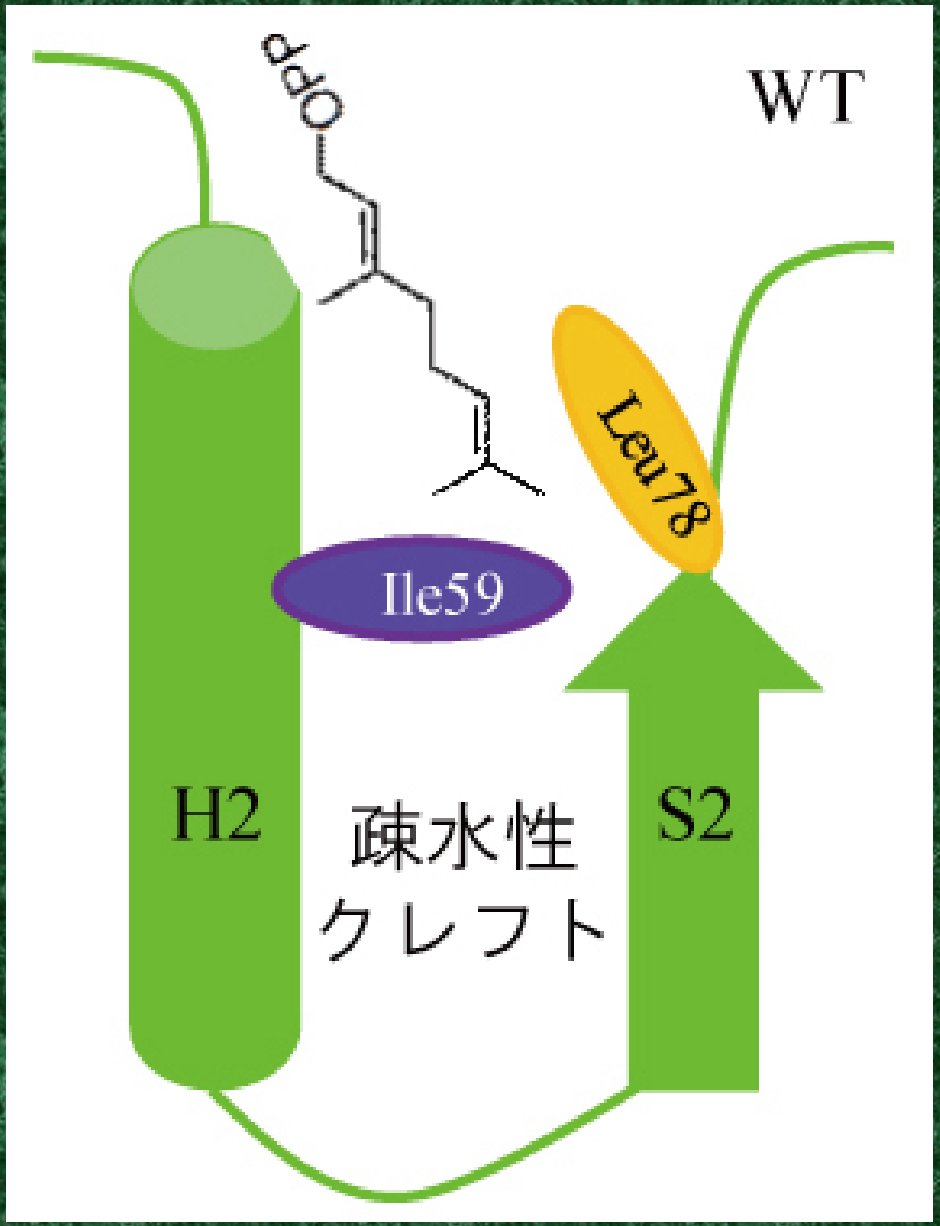
L78A変異体

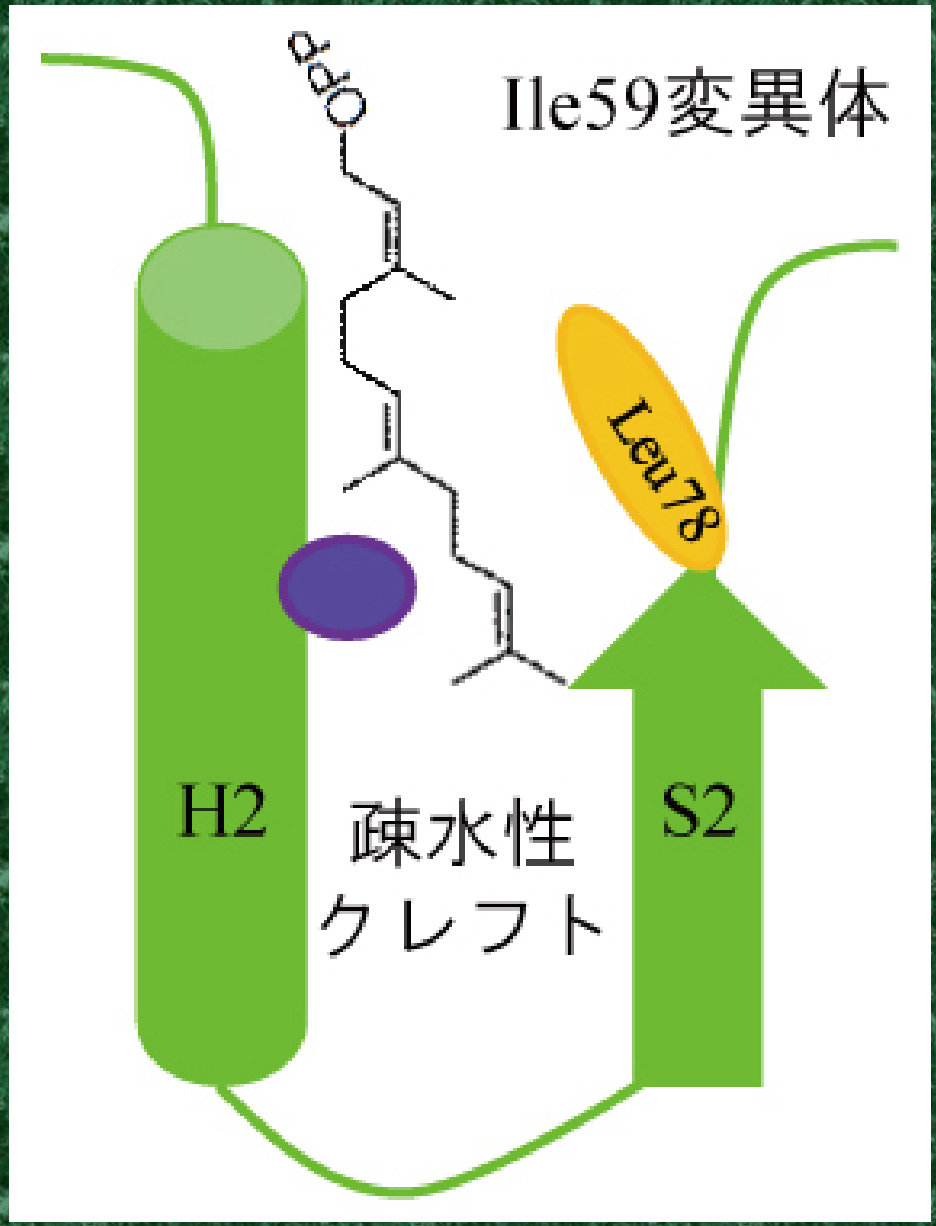


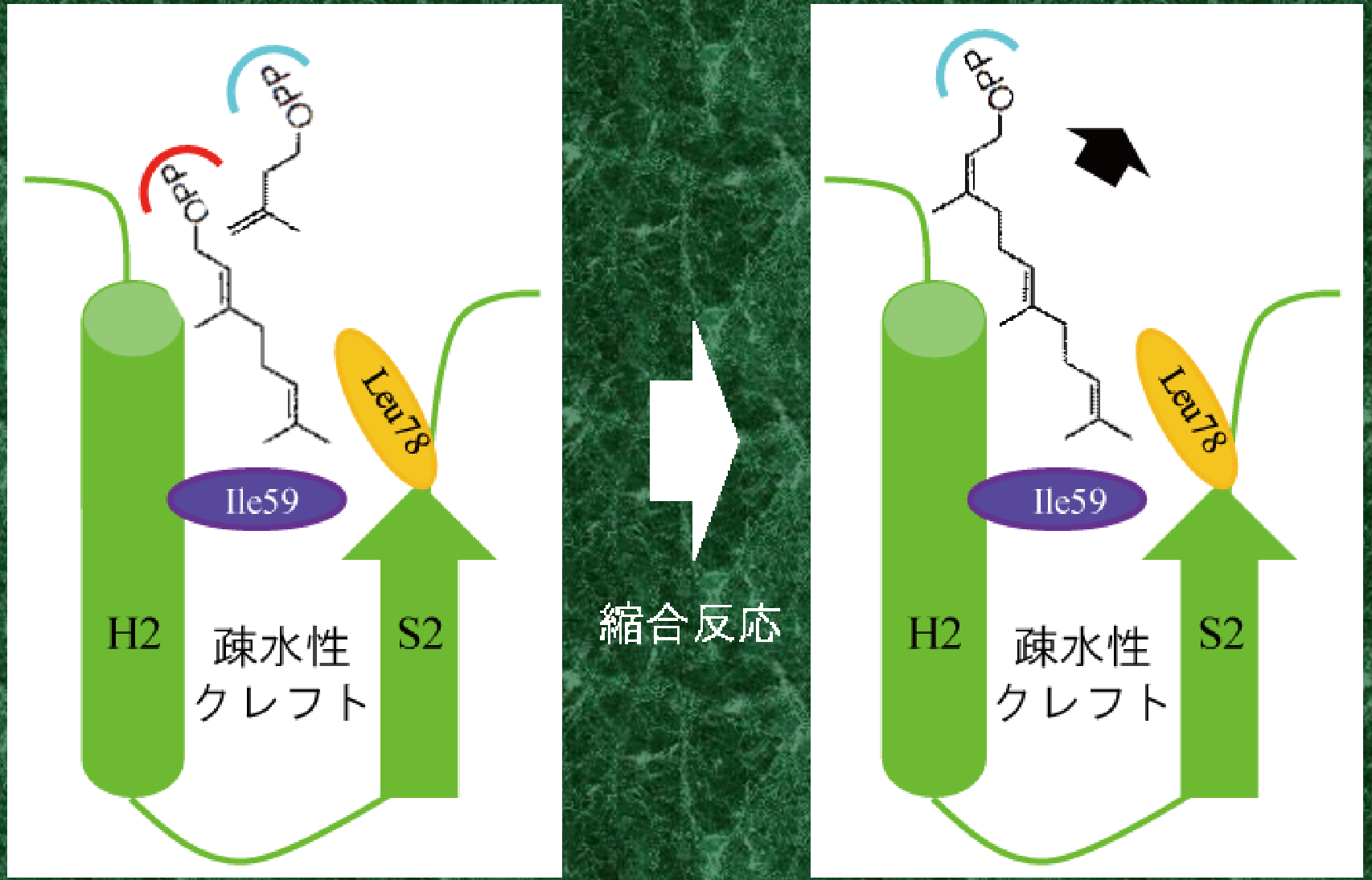
6：WT+GPP
7：L78A+GPP
8：L78A+FPP

展開溶媒
MeOH:Acetone=8:2

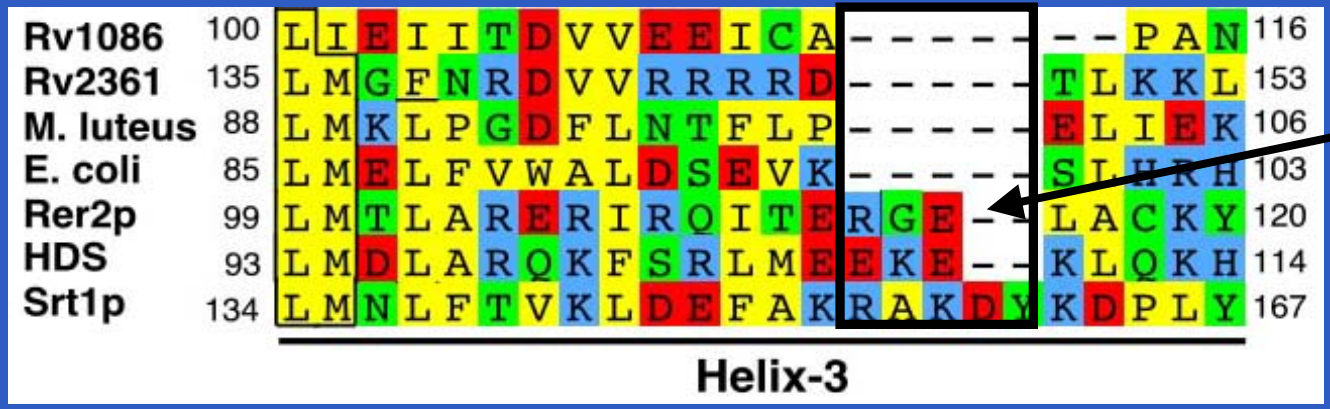
Ambo, T. et al., *Biochem. Biophys. Res. Commun.*, 375, 436-440 (2008)

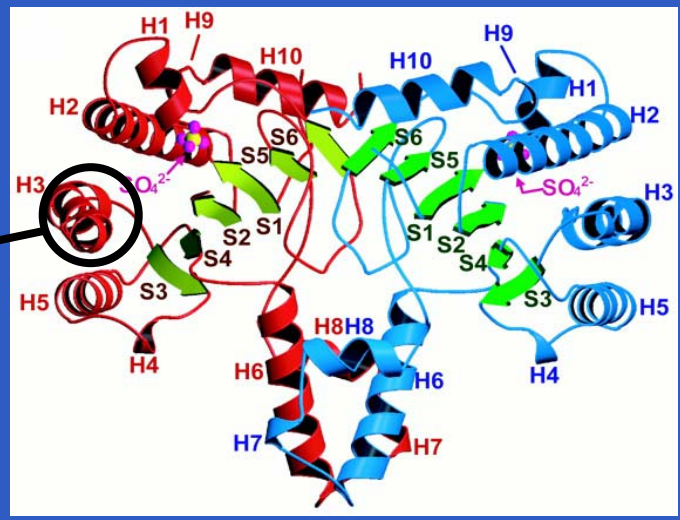

WT
OPP
Leu78
Ile59
H2
疎水性
クレフト
S2


Ile59変異体
OPP
Leu78
H2
疎水性
クレフト
S2


OPP
OPP
Leu78
Ile59
H2
疎水性
クレフト
S2
縮合反応
OPP
Leu78
Ile59
H2
疎水性
クレフト
S2

Characteristic Region in Long-Chain *cis*-Prenyltransferase





M. Luteus B-P 26 UPP synthase

Fujihashi *et al. Proc. Natl. Acad. Sci*. 4337-42 (2001)

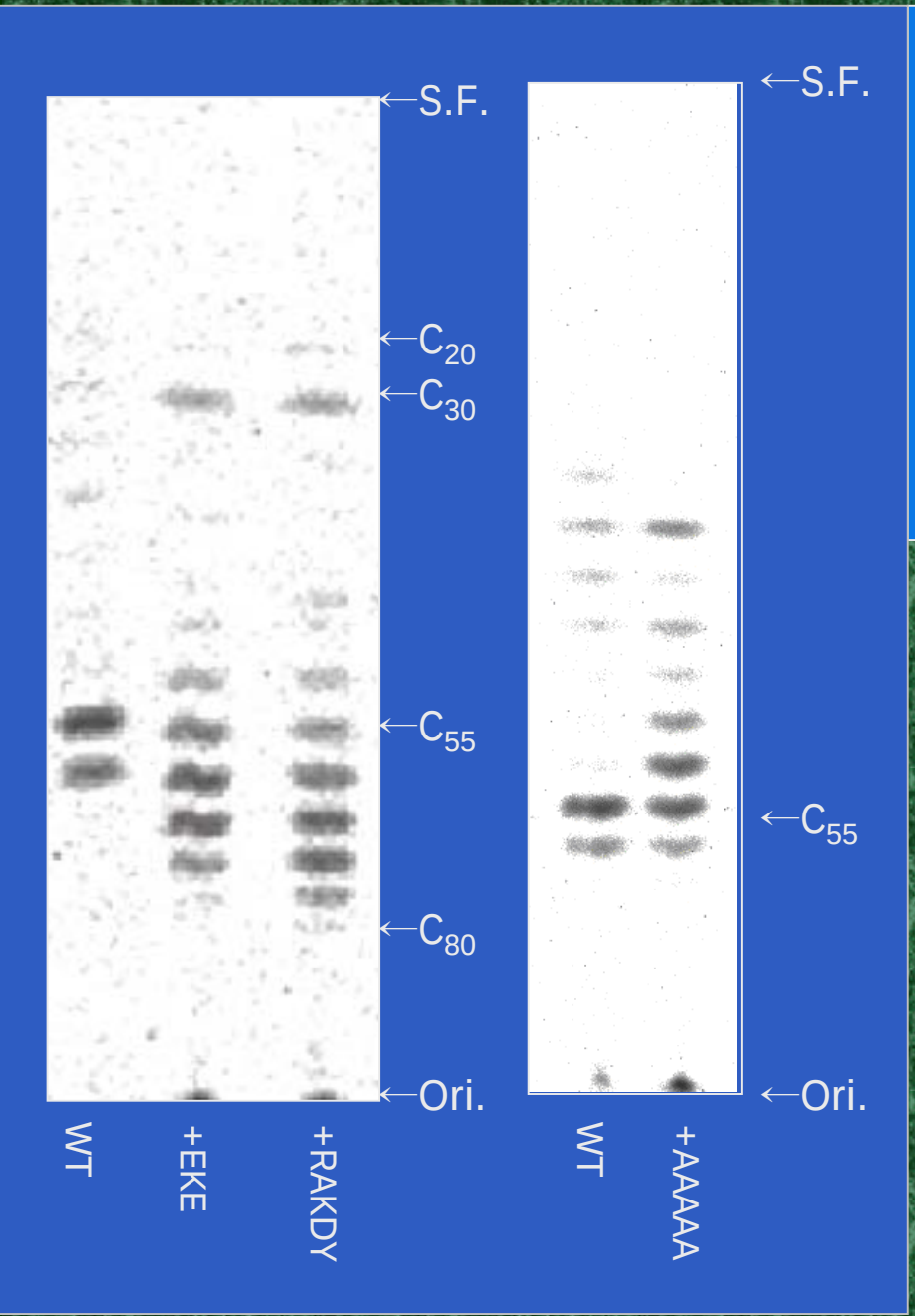
M. luteus UPS

- UPS WT 99- FLP ELI -104
- +EKE 99- FLP EKE ELI -104
- +RAKDY 99- FLPRAKDYELI -104
- +AAAAA 99- FLPAAAAAELI -104

Yeast Srt1p

- Srt1p WT 145- FAKRAKDYKDP -155
- Srt1p delta 145- FAK ----- KDP -155

Product Analysis of Mutant Enzymes



←***M. luteus*** **UPS mutant**

Reversed phase TLC

solvent･･･Acetone/water(19:1)

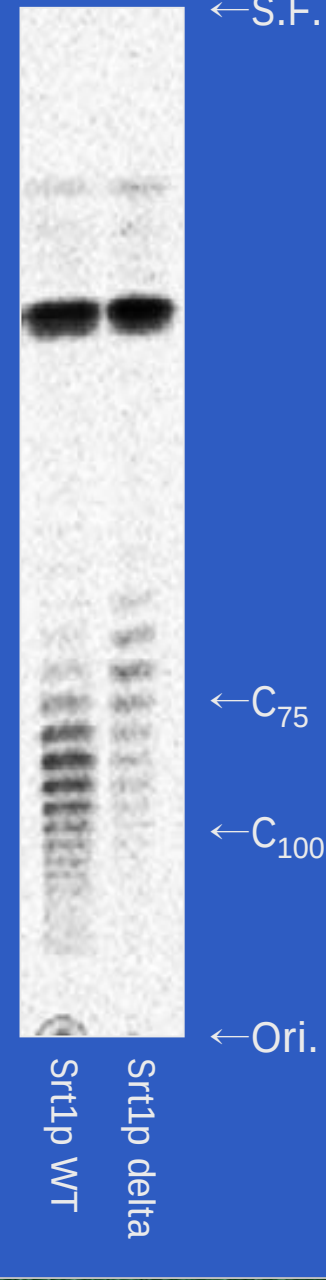
- UPS WT 99 FLP ELI 104
- +EKE 99 FLP **EKE** ELI 104
- +RAKDY 99 FLP**RAKDY**ELI 104
- +AAAAA 99 FLP**AAAAA**ELI 104

Yeast Srt1p mutant→

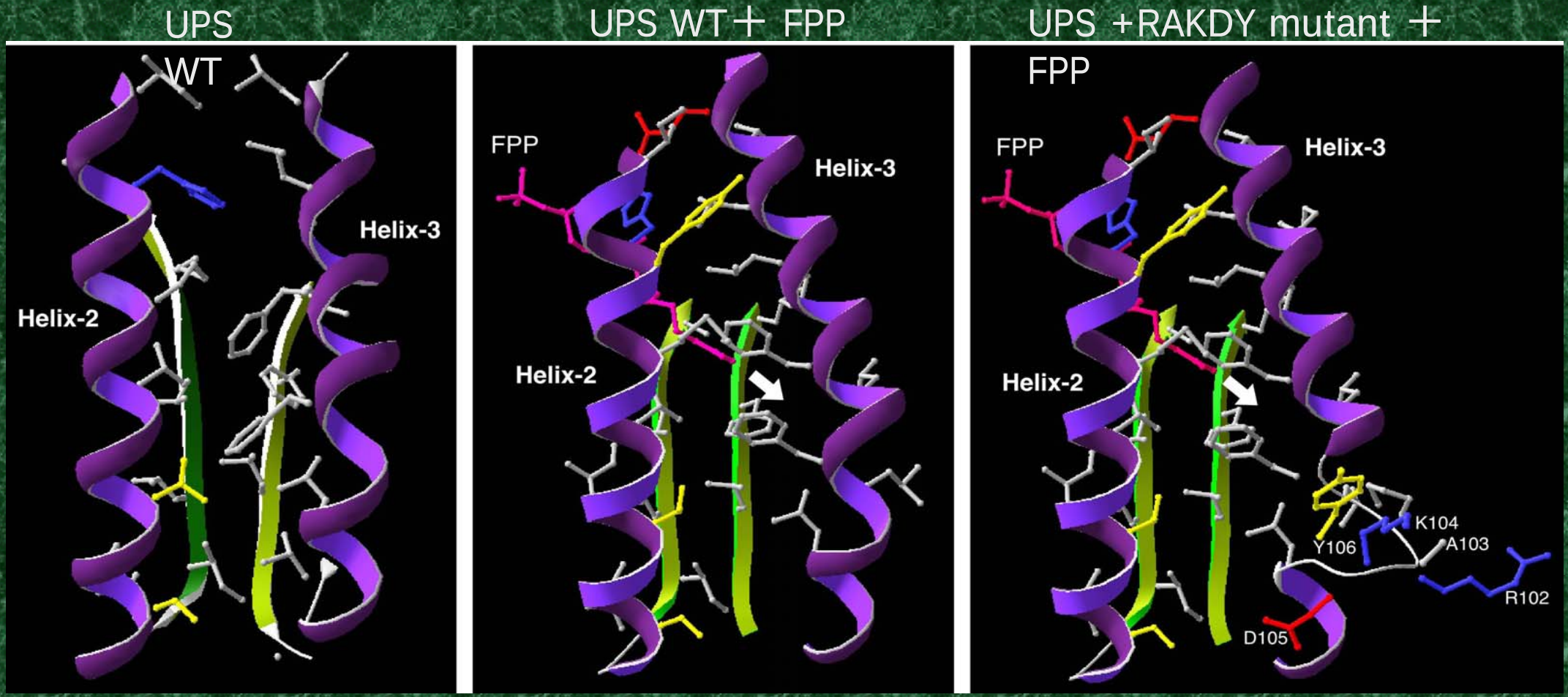
Reversed phase TLC

solvent･･･Acetone/water(39:1)

- Srt1p WT 145 FAK**RAKDY**KDP 155
- Srt1p delta 145 FAK ----- KDP 155



Structure model of the +RAKDY mutant



- Insertion of charged amino acid residues at Helix-3

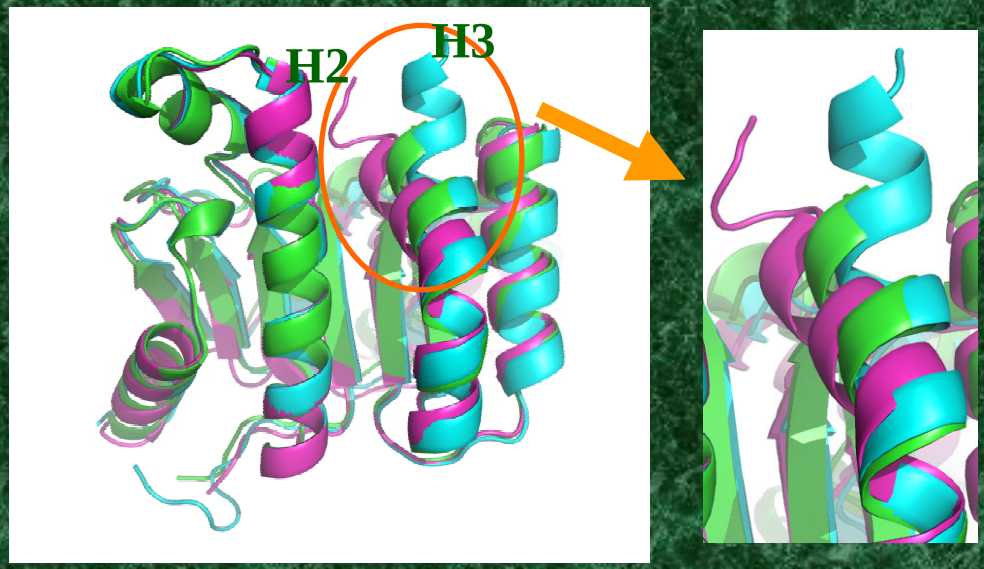
・・・side wall of large hydrophobic cleft

⇒Insertion of charged residues may **control** the bending direction of the elongating product.

Medium-chain typeにおけるHelix3の柔軟性

UPS（Medium-chain type）

M. luteus B-P 26 UPS (Fujihashi *et al.* (2001))



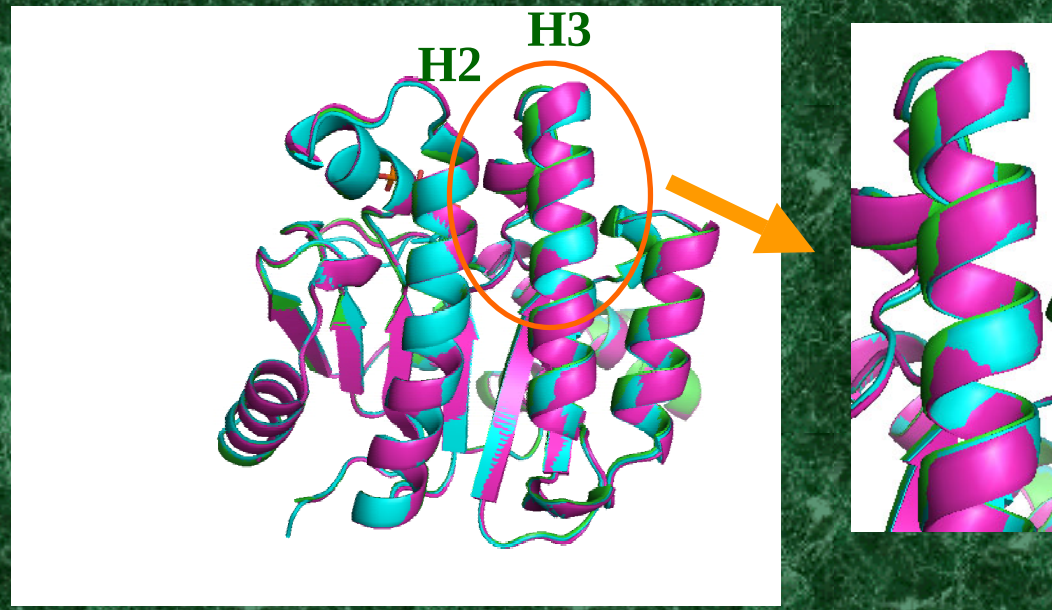
1F75 (subunit A)

1F75 (subunit B)

福地さと子 修士論文 (2007)

cis-FPS（Short-chain type）

M. tuberculosis (Wang *et al.* (2008))

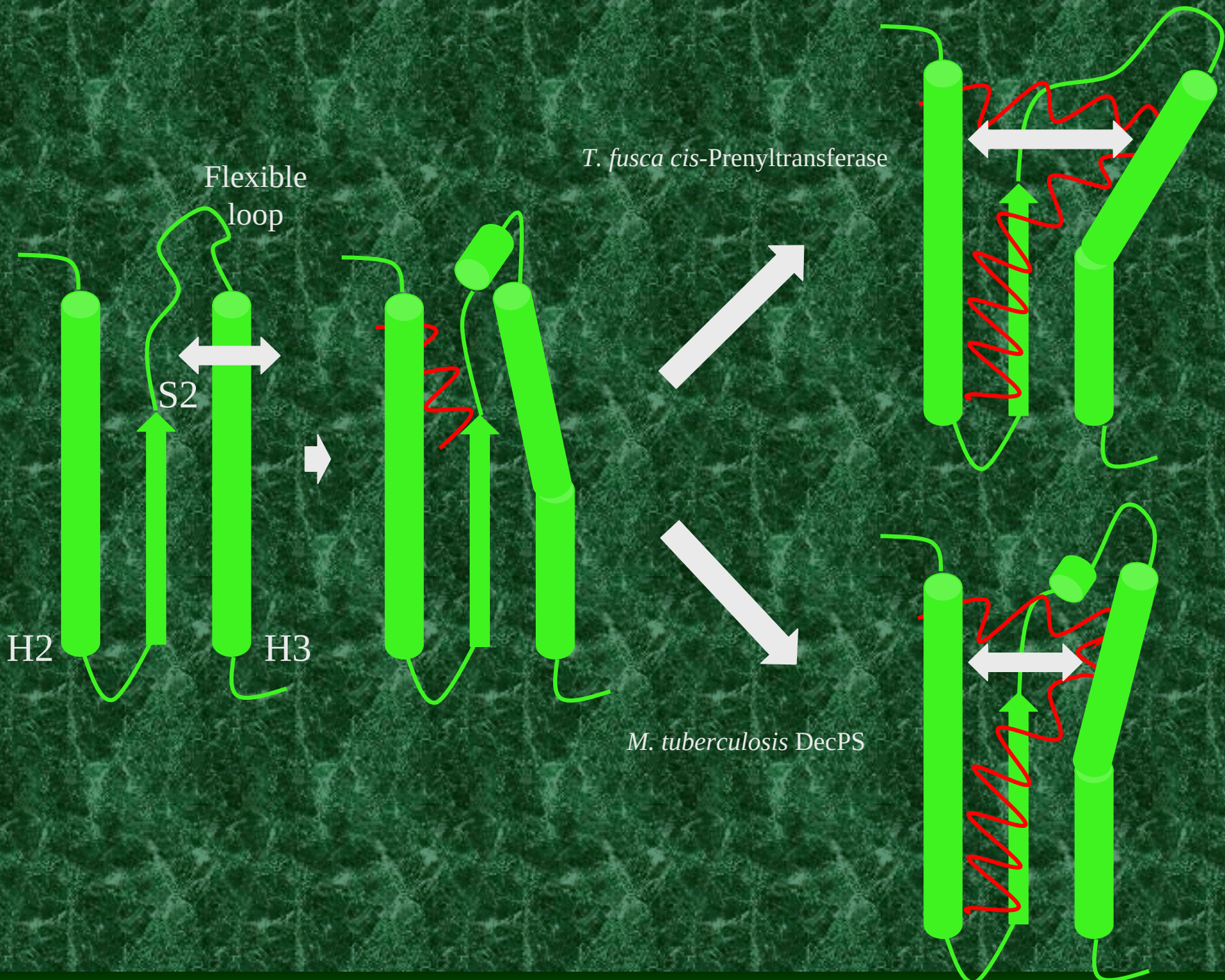


2VFW: apo型

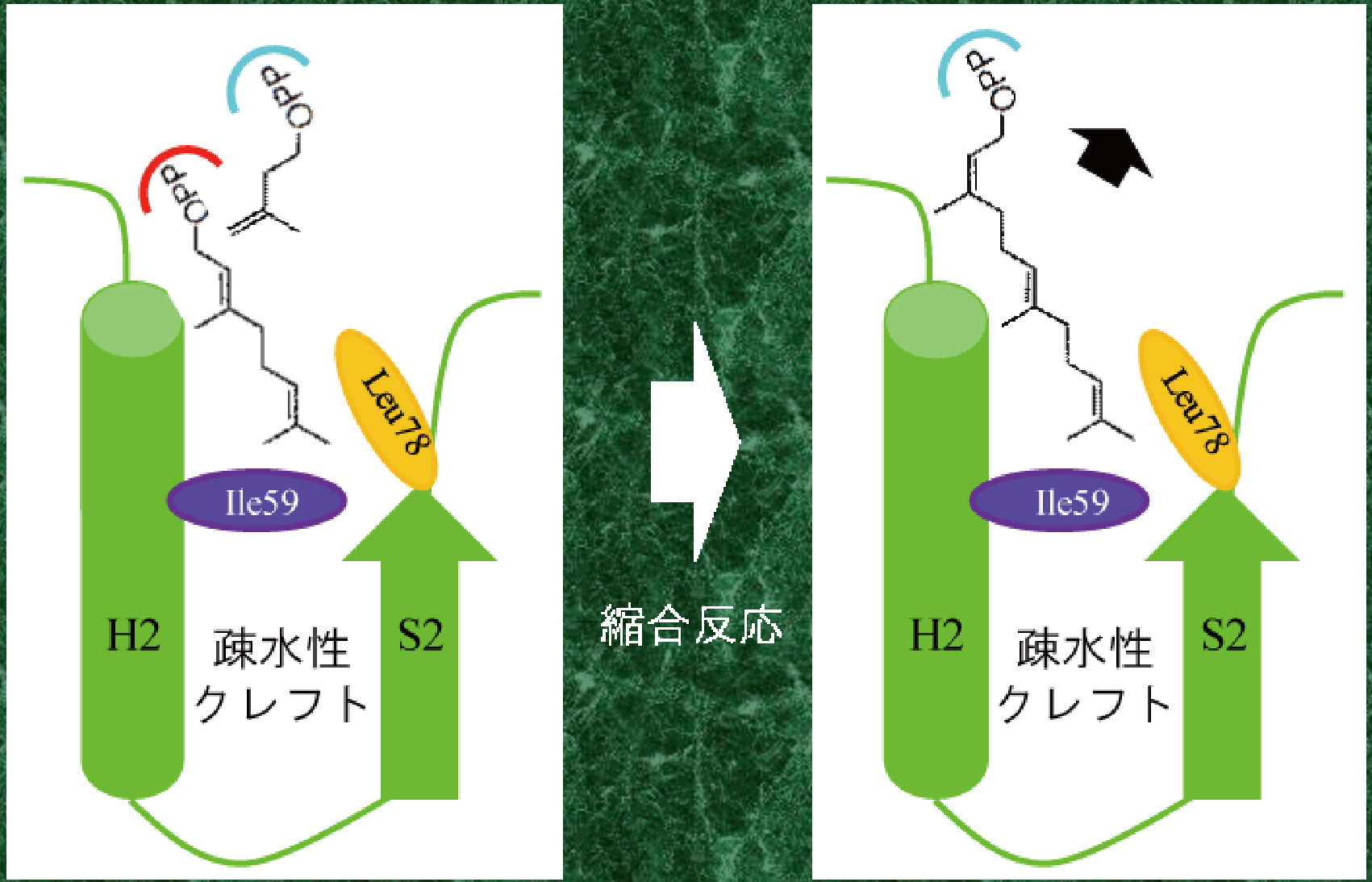
2VG1: FPP結合型

2VG0: GPP analog結合型

Medium-chain *cis*-Prenyltransferaseの鎖長制御機構



Short-chain *cis*-Prenyltransferaseの鎖長制御機構



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